520 Rec'd PCT/PTO 29 NOV 1999

FORM PTO-1390 (REV 11-98) U S DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

ATTORNEY'S DOCKET NUMBER: Bayer 10,203-KGB

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

U.S. APPLICATION NO. (If known, see 37 CFR 1.5): 09/424686

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/EP98/03468	09 June 1998 (09.06.98)	20 June 1997(20.06.97) 14 April 1998(14.04.98) 26 March 1998(26.03.98)

TITLE OF INVENTION: HUMAN CATALYTIC TELOMERASE SUB-UNIT AND ITS DIAGNOSTIC AND THERAPEUTIC USE

APPLICANT(S) FOR DO/EO/US

SEE ATTACHED APPENDIX

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. [X] This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. [ ] This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. [X] This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. [X] A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. [X] A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   - a. [ ] is transmitted herewith (required only if not transmitted by the International Bureau).
   - b. [X] has been transmitted by the International Bureau.
   - c. [ ] is not required, as the application was filed in the United States Receiving Office (RO/US).
6. [X] A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. [ ] Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   - a. [ ] are transmitted herewith (required only if not transmitted by the International Bureau).
   - b. [ ] have been transmitted by the International Bureau.
   - c. [ ] have not been made; however, the time limit for making such amendments has NOT expired.
   - d. [ ] have not been made and will not be made.
8. [ ] A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. [X] An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. [ ] A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern document(s) or information included:

11. [ ] An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. [X] An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. [X] A FIRST preliminary amendment.
    [ ] A SECOND or SUBSEQUENT preliminary amendment.
14. [ ] A substitute specification.
15. [ ] A change of power of attorney and/or address letter.
16. [X] Other items or information:

    a) Copy of the WO-Publication (WO 98/59040)
    b) Copy of the PCT-Request (PCT/RO/101)
    c) Copy of the search report (PCT/ISA/210) in English and German
    d) Copy of the examination report (PCT/IPEA/409) in German
    e) Appendix with Express Mail Label No.
    f) Figs. 1-19
    g) Notice of Change of Firm Name

420 Rec'd PCT/PTO 29 NOV 1999

U.S. APPLICATION NO (if known, see 37 CFR 1.5)	INTERNATIONAL APPLICATION NO	ATTORNEY'S DOCKET NUMBER
09/424686	PCT/EP98/03468	Bayer 10,203-KGB

17. [X] The following fees are submitted:

BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :

	CALCULATIONS	PTO USE ONLY
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO **$970.00**		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO....... **$840.00**		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO **$760.00**		
International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4) **$670.00**		
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4) **$96.00**		
ENTER APPROPRIATE BASIC FEE AMOUNT =	$ 840.00	
Surcharge of **$130.00** for furnishing the oath or declaration later than [ ] 20 [ ] 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	13 - 20 =		X **$18.00**	$	
Independent claims	1 - 3 =		X **$78.00**	$	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ **$260.00**	$	

TOTAL OF ABOVE CALCULATIONS =	$ 840.00	
Reduction of 1/2 for filing by small entity, if applicable. A Small Entity Statement must also by filed (Note 37 CFR 1.9, 1.27, 1.28).	$	
SUBTOTAL =	$ 840.00	
Processing fee of **$130.00** for furnishing the English translation later than [ ] 20 [ ] 30 months from the earliest claimed priority date (37 CFR 1.492(f)). +	$	
TOTAL NATIONAL FEE =	$ 840.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property +	$	
TOTAL FEES ENCLOSED =	$ 840.00	
	Amount to be: refunded	$
	charged	$ 840.00

a. [ ] A check in the amount of $________ to cover the above fees is enclosed.

b. [X] Please charge my Deposit Account No. 14-1263 in the amount of $ 840.00 to cover the above fees. A duplicate copy of this sheet is enclosed.

c. [X] The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 14-1263. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

Kurt G. Briscoe, Esq.
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SIGNATURE:

Kurt G. Briscoe
NAME

33,141
REGISTRATION NUMBER

09/424686

420 Rec'd PCT/PTO 29 NOV 1999

Attorney Docket No. :Bayer 10,203-KGB:pb
:Le A 32 486-US KK/Sto/gp

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants	:	GUSTAV HAGEN ET AL.
Serial No.	:	TO BE ASSIGNED
Filed	:	HEREWITH
For	:	HUMAN CATALYTIC TELOMERASE SUB-UNIT AND ITS DIAGNOSTIC AND THERAPEUTIC USE
Art Unit	:	TO BE ASSIGNED
Examiner	:	TO BE ASSIGNED

November 29, 1999

Hon. Assistant Commissioner
for Patents
Washington, D. C. 20231

PRELIMINARY AMENDMENT

Sir:

Prior to examination, please amend the above-identified application as follows:

IN THE CLAIMS:

Claim 3, delete "compounds" and substitute -- telomerase --; and delete "Claims" and substitute -- Claim --; and delete "and 2".

Claim 5, after "3" insert a period and cancel the balance of the claim.

Claim 6, delete “Claims 1 and 2,” and substitute -- Claim 1--.

Claims 7-9, delete “Use of” and substitute -- Method of using --.

Claim 7, delete “Claims 3 and 4” and substitute -- Claim 3 --.

Claim 10, delete “Claims 3 and 4” and substitute --Claim 3 --.

Claim 12, delete “Claims 1 and 2” and substitute -- Claim 1 --.

Claim 13, delete “the telomerase according to Claims 1 and 2” and substitute -- a telomerase --.

REMARKS

The foregoing amendments remove multiple dependencies and otherwise place the claims in better form for U.S. examination.

Early and favorable action is earnestly solicited.

Respectfully submitted,
NORRIS McLAUGHLIN & MARCUS

By______________________________
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- 1 - 420 Rec'd PCT/PTO 29 NOV 1999

Catalytic subunit of human telomerase and its diagnostic and therapeutic use

Structure and function of the chromosome ends

The genetic material of eukaryotic cells is distributed on Linear chromosomes. The ends of these hereditary units are termed telomeres, derived from the Greek words *telos* (end) and *meros* (part or segment). Most telomeres consist of repeats of short sequences which are mainly constructed from thymine and guanine (Zakian, 1995). The telomere sequences of related organisms are often similar and these sequences are even conserved between species which are more phylogenetically remote. It is a remarkable fact that the telomeres are constructed from the sequence TTAGGG in all the vertebrates which have so far been examined (Meyne *et al.*, 1989).

The telomeres exert a variety of important functions. They prevent the fusion of chromosomes (McClintock, 1941) and consequently the formation of dicentric hereditary units. Chromosomes of this nature, possessing two centromeres, can lead to the development of cancer due to loss of heterozygosity or the duplication or loss of genes.

In addition, telomeres serve the purpose of distinguishing intact hereditary units from damaged hereditary units. Thus, yeast cells ceased dividing when they harboured a chromosome which lacked a telomere (Sandell and Zakian, 1993).

Telomeres carry out another important task in association with DNA replication in eukaryotic cells. In contrast to the circular genomes of prokaryotes, the Linear chromosomes of eukaryotes cannot be completely replicated by the DNA polymerase complex. RNA primers are required for initiating DNA replication. After the RNA primers have been eliminated and the Okazaki fragments have been extended and then ligated, the newly synthesized DNA strand lacks the 5' end because the RNA primer at that point cannot be replaced with DNA. For this reason, without special protective mechanisms, the chromosomes would shrink with every cell division ("end-replication problem", Harley *et al.*, 1990). The non-coding telomere

sequences probably represent a buffer zone for preventing the loss of genes (Sandell and Zakian, 1993).

Over and above this, telomeres also play an important role in regulating cell ageing (Olovnikov, 1973). Human somatic cells exhibit a limited capacity to replicate in culture; after a certain time they become senescent. In this condition, the cells no longer divide even after being stimulated with growth factors; however, they do not die but remain metabolically active (Goldstein, 1990). Various observations provide support for the hypothesis that a cell determines from the length of its telomeres how often it can still divide (Allsopp *et al.*, 1992).

In summary, the telomeres consequently possess central functions in the ageing of cells and in the stabilization of the genetic material and prevention of cancer.

The enzyme telomerase synthesizes the telomeres

As described above, organisms possessing Linear chromosomes are only able to replicate their genomes incompletely in the absence of a special protective mechanism. Most eukaryotes use a special enzyme, i.e. telomerase, to regenerate the telomere sequences. Telomerase is expressed constitutively in the single-cell organisms which have so far been examined. By contrast, in humans, telomerase activity was only detected in germ cells and tumour cells whereas neighbouring somatic tissue did not contain any telomerase (Kim *et al.*, 1994).

Telomerase in ciliates

Like the telomeres, telomerase was identified for the first time in the ciliate *Tetrahymena thermophila*. Telomerase activity was detected by extending the single-stranded oligonucleotide d(TTGGGG)$_4$ in the presence of dTTP and dGTP (Greider and Blackburn, 1985). In this reaction, the *Tetrahymena* telomere sequence TTGGGG was added repeatedly to the primer. Even when an oligonucleotide having the irregular telomere sequence of *Saccharomyces cerevisiae*, T(G)$_{1-3}$, was offered as the starting material, the telomerase

extended the primer with the telomere sequence of *Tetrahymena* (Greider and Blackburn, 1985). From these results, it was concluded that the telomerase itself carries the template for the sequence of the telomeres.

Once the existence of an RNA component in the telomerase had initially been demonstrated (Greider and Blackburn, 1987), the gene for the RNA subunit of the telomerase was cloned a short while later (Greider and Blackburn, 1989). This RNA contains a region which is complementary to the *Tetrahymena* telomere sequence (termed "complementary region" below). The activity of the telomerase depended on the RNA component, as was demonstrated by digesting the RNA, leading in turn to subsequent loss of activity. If the complementary region of the telomerase RNA was mutated, the corresponding mutations were incorporated *in vivo* into the *Tetrahymena* telomeres (Yu *et al.*, 1990). Telomerase consequently belongs to the class of RNA-dependent DNA polymerases.

The first protein subunits of the *Tetrahymena* telomerase, i.e. p80 and p95, were identified in 1995 (Collins *et al.*, 1995). The observation that p95 anchors the enzyme to the DNA and p80 binds the RNA component led to the following model: the telomerase RNA anneals by its complementary region to the single-stranded 3' overhang. The 3' overhang is extended by incorporating the corresponding nucleotides in the 5'-3' direction. The *de novo* synthesis of telomeres probably involves an elongation step and a translocation step. Once a telomere sequence has been synthesized, the telomerase presumably moves along the DNA until it is once again in a position to be able to add a complete telomere sequence. This model does not have to be generally valid since great differences exist between the telomerases of different species with regard to the number of nucleotides which the enzyme adds before it dissociates from the telomere (Prowse *et al.*, 1993).

In addition to this, telomerase subunits from other organisms have also recently been identified. Two protein subunits, i.e. p123 and p43, which do not exhibit any homology with the *Tetrahymena* telomerase proteins, have been found in the ciliate *Euplotes aediculatus*. The telomerase subunit p123 exhibits a basic domain at its N terminus and a domain for a reverse transcriptase (RT) at the C terminus, suggesting this protein has a catalytic function,

(Lingner *et al.*, 1997). Furthermore, p123 has been reported to share significant homology with the *Saccharomyces cerevisiae* protein Est2 which was found by Lundblad (Lingner *et al.*, 1997).

Whereas p80 and p95 have not hitherto been demonstrated to possess any function which is essential for telomerase activity, the potential catalytic telomerase subunits p123/est2p have been unambiguously shown to have a key function: mutation of the active centre of the est2p RT led to significant truncation of the telomeres in yeast cells (Lingner *et al.*, 1997).

Telomerase components from mammalian cells

The RNA components of the telomerases of various organisms, inter alia of *Saccharomyces cerevisiae*, mice and humans (Singer and Gottschling, 1994; Blasco *et al.*, 1996; Feng *et al.*, 1995), have by now been cloned. All the telomerase RNAs known to date comprise a region which is complementary to the telomere sequence of a particular organism. However, the primary sequence of the human telomerase RNA (hTR) does not display any similarity to the RNA components of the ciliates or of *Saccharomyces cerevisiae*. On the other hand, regions exist which are conserved between human and murine telomerase RNA (Feng *et al.*, 1995).

The isolation of a human telomerase-associated protein (hTP1) has recently been described (Harrington *et al.*, 1997). On the basis of its homology with the *Tetrahymena* telomerase p80 subunit, the corresponding gene was found in an EST data base which is not available to the general public (Harrington *et al.*, 1997). hTP1 is composed of 2627 amino acids and, in the N-terminus, exhibits three domains which possess at most 46% homology with p80. 16 repeats of the amino acids tryptophan and asparagine, which presumably mediate a protein/protein interaction, were shown to be present, as an additional structural element, in the C-terminal region.

Activation of the telomerase in human tumours

In humans, it was originally only possible to demonstrate telomerase activity in germ line cells and not in normal somatic cells (Hastie *et al.*, 1990; Kim *et al.*, 1994). After a more sensitive detection method had been developed (Kim *et al.*, 1994) a low level of telomerase activity was also detected in hematopoietic cells (Broccoli *et al.*, 1995; Counter *et al.*, 1995; Hiyama *et al.*, 1995). However, these cells nevertheless exhibited a reduction in the telomeres (Vaziri *et al.*, 1994; Counter *et al.*, 1995). It has still not been clarified whether the quantity of enzyme in these cells is insufficient to compensate for the telomere loss or whether the measured telomerase activity stems from a subpopulation, e.g. of incompletely differentiated $CD34^{+}38^{+}$ precursor cells (Hiyama *et al.*, 1995). In order to clarify this point, it would be necessary to detect the telomerase activity which was present in a single cell.

Interestingly enough, however, significant telomerase activity has been detected in a large number of the tumour tissues which have been tested to date (1734/2031, 85%; Shay, 1997), whereas no activity has been found in normal somatic tissue (1/196, <1%, Shay, 1997). In addition, a variety of investigations demonstrated that the telomeres continued to shrink in senescent cells which were transformed with viral oncoproteins and that it was only possible to find telomerase in the subpopulation which survived the growth crisis (Counter *et al.*, 1992). The telomeres were also stable in these immortalized cells (Counter *et al.*, 1992). Similar findings derived from investigations in mice (Blasco *et al.*, 1996) support the assumption that reactivation of the telomerase is a late event in tumorigenesis.

Based on these results, a "telomerase hypothesis" was developed which links the loss of telomere sequences and cell ageing to telomerase activity and the genesis of cancer. In long-lived species such as humans, the shrinking of the telomeres can be regarded as a tumour suppression mechanism. Differentiated cells, which do not contain any telomerase, cease dividing when the telomeres have reached a particular length. If such a cell mutates, a tumour can only develop from it if the cell is able to extend its telomeres. Otherwise, the cell would continue to lose telomere sequences until its chromosomes became unstable and it finally died. Reactivation of the telomerase is presumably the main mechanism which tumour cells deploy in order to stabilize their telomeres.

It follows from these observations and ideas that it should be possible to develop a therapy for tumours based on inhibiting telomerase activity. Conventional cancer therapies using cytostatic agents or short-wave irradiation damage all the dividing cells in the body in addition to damaging the tumour cells. However, since it is only germ line cells which contain significant telomerase activity, apart from tumour cells, telomerase inhibitors would attack the tumour cells more specifically and consequently evoke fewer undesirable side effects. Since telomerase activity has been detected in all the tumour tissues tested to date, it would be possible to employ these therapeutic agents against all types of cancer. The effect of telomerase inhibitors would then set in when the telomers of the cells had shortened to such an extent that the genome had become unstable. Since tumour cells usually exhibit shorter telomeres than do normal somatic cells, it would be cancer cells which would first of all be eliminated by telomerase inhibitors. By contrast, cells possessing long telomeres, such as the germ cells, would not be damaged until a much later stage. Telomerase inhibitors consequently represent an approach which points the way forward for cancer therapy.

However, it will only be possible to provide unambiguous answers to questions regarding the nature and the points of attack of physiological telomerase inhibitors when the protein structures of the enzyme, together with their functions, have also been identified and a deeper understanding of the various telomere-binding proteins has been obtained.

The invention relates to the catalytically active human telomerase subunit (phTC), where appropriate in purified form, to active moieties of the protein, to modulators, in particular agonists of the protein, to substances which imitate the function of the protein and to combinations of these components.

The invention furthermore relates to:

- The nucleic acid sequence which encodes the human protein phTC, specifically:
    - the genomic sequence of the hTC gene,
    - the cDNA sequence of the hTC gene,

- the DNA sequence of hTC variants
- the sequence of the mRNA which is transcribed from the hTC gene,
- parts of the abovementioned sequences, including the DNA sequence (SEQ ID No. 1) of hTC which is shown in Fig. 1.

- The nucleic acid sequences which encode hTC-homologous proteins in other mammals, specifically:

    - the genomic sequences of hTC-homologous genes,
    - the cDNA sequences of hTC-homologous genes,
    - the sequences of the mRNAs which are transcribed from hTC-homologous genes,
    - parts of the abovementioned sequences.

- Nucleic acid sequences which, in humans and other mammals, encode proteins which are related to the phTC protein, specifically:

    - the genomic sequences of hTC-related genes in humans and other mammals,
    - the cDNA sequences of hTC-related genes in humans and other mammals,
    - the sequences of the mRNAs which are transcribed from hTC-related genes in humans and other mammals,
    - parts of the abovementioned sequences.

- The above-described phTC protein, which is isolated from mammalian cells (cf. Fig. 2 and SEQ ID No. 2).

- The phTC protein which is labelled with a detection reagent, with the detection reagent preferably being an enzyme, a radioactively labelled element or a fluorescent chemical.

- An antibody which is directed against the phTC protein.

According to a preferred embodiment, this antibody is a polyclonal antibody.

According to another preferred embodiment, this antibody is a monoclonal antibody.

Antibodies of this nature can be produced, for example, by injecting a host, which is substantially immunocompetent, with a quantity of a phTC polypeptide, or a fragment thereof, which is effective for producing the antibody, and by subsequently isolating this antibody.

In addition, an immortalized cell line which produces monoclonal antibodies can be obtained in a manner known per se.

Where appropriate, the antibodies can be labelled with a detection reagent.

Fragments which possess the desired specific binding properties can also be employed instead of the complete antibody.

Preferred examples of such a detection reagent are enzymes, radioactively labelled elements, fluorescent chemicals or biotin.

- Oligonucleotides in purified form which have a sequence which is identical or exactly complementary to a contiguous sequence, of from 10 to 500 nucleotides in length, of the above-described genomic DNA, cDNA or mRNA.

An oligonucleotide of this nature can, in particular, be an oligodeoxy-ribonucleotide or an oligoribonucleotide or a peptide nucleic acid (PNA).

Preference is given to oligonucleotides which inhibit, repress or block the activity of the telomerase when they bind to the hTC mRNA.

- A DNA sequence, or a degenerate variation of this sequence, which encodes the phTC protein, or a fragment of this protein, where appropriate comprising the DNA sequence in Figure 1a, or a DNA sequence which hybridizes with the previously cited DNA sequence under standard hybridization conditions.

- A recombinant DNA molecule which comprises a DNA sequence, or a degenerate variation of this sequence, which encodes phTC or a fragment of phTC, with the latter sequence preferably comprising the DNA sequence in Figure 1a, or which comprises a DNA sequence which hybridizes with the previously cited DNA sequence under standard hybridization conditions.

In the abovementioned recombinant DNA molecule, the described DNA is preferably linked to an expression control sequence.

Examples of expression control sequences which are particularly preferred are the early or late promoter of the SV40 virus or adenovirus, the lac system, the trp system, the TAC system, the TRC system, the main operator and promoter regions of phage λ, the control regions of the fd coat protein, the 3-phosphoglycerate kinase promoter, the acid phosphatase promoter and the yeast α-mating factor promoter.

- A single-cell host which has been transformed with the above-described recombinant DNA molecule which comprises the DNA sequence, or a degenerate variation of this sequence, which encodes the phTC protein or a part of this protein. In this recombinant DNA molecule, the said DNA sequence is linked to an expression control sequence.

Preferred examples of the single-cell host are: *E. coli, Pseudomonas, Bacillus, Streptomyces*, yeasts, CHO, R1.1, B-W, L-M, COS 1, COS 7, BSC1, BSC40 and BMT10 cells, plant cells, insect cells and mammalian cells in cell culture.

- A recombinant virus which is transformed with one of the previously described DNA molecules or a derivative or fragment of this molecule.

- A method for inhibiting telomerase activity in human cells, preferably neoplastic cells, in which an exogenous polynucleotide which consists of a transcription unit is transferred into the cells. This transcription unit comprises a polynucleotide sequence of at least 29 consecutive nucleotides, which sequence is substantially identical or substantially complementary to the hTC RNA sequence and is linked to a heterologous transcription-regulating sequence which controls the transcription of the linked polynucleotide in the said cells.

Preferably, the abovementioned heterologous transcription-regulating sequence comprises a promoter which is constitutively active in human cells.

Alternatively, the heterologous transcription-regulating sequence can comprise a promoter which can be induced or repressed in human cells by adding a regulatory substance. Examples of such promoters are inducible and repressible tetracycline-dependent promoters, heat shock promoters and metal ion-dependent promoters.

The abovementioned exogenous polynucleotide can, for example, be a viral genome containing a transcription unit from the human hTC DNA component.

Particularly preferably, the said transcription unit produces antisense RNA which is substantially complementary to the human hTC RNA component.

Particular preference is also given to the exogenous polynucleotide being able to comprise the sequence in Fig. 1a.

- A polynucleotide for the genetic therapy of a human disease. This polynucleotide consists of a transcription unit which comprises a polynucleotide sequence of at least 9 consecutive nucleotides, which sequence is substantially identical or substantially

complementary to the hTC RNA sequence and is linked to a heterologous transcription-regulating sequence which controls the transcription of the linked polynucleotide in said cells.

- A method for detecting telomerase-associated conditions in a patient, which method comprises the following steps:

A. Detecting the phTC protein in body fluids or cell samples in order to obtain a diagnostic value;
B. Comparing the diagnostic value with standard values for the phTC protein in standardized normal cells or body fluids of the same type as the test sample;
C. Detecting diagnostic values which are higher or lower than the standard comparative values and which indicate a telomerase-associated condition, which condition in turn indicates a pathogenic condition.

This method is preferably employed for detecting a neoplastic disease in a patient. The method then comprises the following steps:

A. Detecting the phTC protein in cell samples in order to obtain a diagnostic value;
B. Comparing the diagnostic value with standard values for the phTC protein in non-neoplastic cells of the same type as the test sample;
C. Diagnostic values which are clearly higher than standard comparative values indicate a neoplastic condition.

- A method for determining the presence of the phTC protein in a cell or cell sample, which method is based on amplifying an hTC polynucleotide, or hybridizing an hTC polynucleotide, a primer or an hTC-complementary sequence with an hTC polynucleotide.

- A test kit for detecting phTC in cell samples and body fluids, with it being possible, for example, for labelled, immunochemically-reactive components to be: polyclonal

antibodies against phTC, monoclonal antibodies against phTC, fragments of these antibodies or a mixture of these components.

- A method for preventing and/or treating cell disturbance or destruction and/or malfunction and/or other symptoms in humans, which method is based on administering a therapeutically effective quantity of catalytically active human telomerase, its functional equivalents or its catalytically active fragments. It is also possible to conceive of using a substance which promotes the production and/or activity of phTC; a substance which can imitate the activity of phTC; a substance which can inhibit the production and/or activity of phTC, or a mixture of these substances. A specific binding partner can also be employed.

The method is preferably employed for preventing or treating ageing or cancer diseases.

Substances which are able to affect the activity of phTC, i.e. inhibit or promote, are here termed modulators. Such modulators can be found, in a manner known per se, by testing their effect on telomerase activity in a telomerase assay. Examples of telomerase assays are given in Example 15.

Modulators of phTC are of interest for treating diseases which are connected with telomerase. The prevention or treatment of ageing processes or of cancer diseases may, in particular, be mentioned in this context.

- An antisense nucleic acid against the hTC mRNA, which nucleic acid comprises a nucleotide sequence which hybridizes with the said mRNA, with the antisense nucleic acid being an RNA or a DNA.

Preferably, the antisense nucleic acid binds to the start codon of the particular mRNA.

- A recombinant DNA molecule which contains a DNA sequence from which an antisense ribonucleic acid against the hTC mRNA is produced during transcription. This said antisense ribonucleic acid comprises a nucleic acid sequence which can hybridize to the said hTC mRNA.

  A DNA molecule of this nature can be used to prepare a cell line having a reduced expression of phTC by transfecting a phTC-producing cell line with this recombinant DNA molecule.

- A ribozyme which cleaves the hTC mRNA.

  This ribozyme is preferably a *Tetrahymena*-type ribozyme or a hammerhead-type ribozyme.

- A recombinant DNA molecule which contains a DNA sequence whose transcription leads to the production of a ribozyme of this nature.

  This recombinant DNA molecule can be used to transfect a phTC-producing cell line.

- A combination which consists of a pair of human hTC polynucleotide PCR primers, with the primers preferably consisting of sequences which correspond to the sequence of the human hTC mRNA or which are complementary to this sequence.

- A combination which comprises a polynucleotide hybridization probe for the human hTC gene, with the probe preferably comprising at least 29 consecutive nucleotides which correspond to the sequence of the human hTC gene or which are complementary to this sequence.

- Animal models which can be used to investigate telomerase/telomere regulation *in vivo*. Thus, tumour development and ageing can, for example, be directly investigated using knockout animals or transgenic animals.

In the case of proteins or peptides, functional equivalents are those compounds which, while being distinguishable with regard to amino acid sequence, essentially have the same functions.

Known examples of these compounds are isoenzymes or so-called microheterogeneities in proteins.

In the case of the oligonucleic or polynucleic acids, functional equivalents are to be understood as being those compounds which differ in nucleotide sequence but which encode the same protein. The existence of such compounds may be attributed, for example, to the fact that the genetic code is degenerate.

Explanation of the figures:

Fig. 1: cDNA sequence of the catalytic subunit of human telomerase (hTC) (SEQ ID No. 1).

Fig. 2: Amino acid sequence which is deduced from the hTC DNA sequence depicted in Fig. 1 (SEQ ID No. 2).
The DNA sequence depicted in Fig. 1 can be completely translated from Position 64 to Position 3461 into an amino acid sequence. The amino acid residues are depicted in accordance with their single-letter code.

Fig. 3: Ethidium bromide-stained agarose gel containing AA281296 DNA which has been treated in different ways.
The figure shows an ethidium bromide-stained 0.8% agarose gel. Two different DNA size standards are loaded in lanes 1 and 8, with the DNA fragment lengths 3, 2, 0.5 and 0.4 kb being pointed out. The AA281296 DNA in pT7T3D was digested with a restriction enzyme Eco RI/Not I (lane 3), Pst I (lane 6) and Xho 1 (lane 7). Undigested AA281296 DNA in pT7T3D was loaded onto lane 2. 1/10 of a PCR

mixture (1 minute 94°C, 2 minutes at 60°C, 3 minutes at 72°C) with the hTC cDNA in pT7T3D and primers 1 (5' GAGTGTGTACGTC-GTCGAGCTGCTCAGGTC 3') and 4 (5' CACCCTCGAGGTGAGACGCTCGGCC 3') [lane 4] and, especially, with primers 6 (5' GCTCGTAGTTGAGCACGCTGAACAGTG 3') and 7 (5' GCCAAGTTCCTGCACTGGCTGATGAG 3') [lane 5] was applied to lanes 4 and 5.

Fig. 4: Detail from a comparison of the protein sequences of the *Euplotes* p123 (p123) and human (phTC) catalytic telomerase subunits.
The conditions (ktuple, gap penalty and gap length penalty) are listed for the Lipman-Pearson protein comparison, using the Lasergene program software (Dnastar, Inc.), which is depicted in this figure. The amino acid residues are depicted in accordance with their single-letter code. The amino acids which are identical between *Euplotes aediculatus* p123 and the identified EST_{+1} are also highlighted using the corresponding letter from the single-letter code. Amino acids which are not identical but whose function is similar or comparable are marked by a :.

Fig. 5: Part of a comparison of the protein sequences of the catalytic telomerase subunits of *Euplotes* p123 (p123), and yeast (est2p).
The condition (Ktuple, gap penalty and gap length penalty) are listed for the Lipman-Pearson protein comparison using Lasergene program software (Dnastar, Inc.) which is dipicted in this figure. The amino acid residues are shown in accordance with their single letter code. The amino acids which are identical between *Euplotes aediculatus* p123 and yeast est2p are likewise given prominence by the corresponding letter from the single-letter code. Amino acids which are not identical, but which are similar or comparable in function, are marked with a :.

Fig. 6: Detail from a comparison of the protein sequences of the yeast (est2p) and human (phTC) catalytic telomerase subunits.

The conditions (ktuple, gap penalty and gap length penalty) are listed for the Lipman-Pearson protein comparison, using the Lasergene program software (Dnastar, Inc.), which is depicted in this figure. The amino acid residues are depicted in accordance with their single-letter code. The amino acids which are identical between yeast est2p and the identified EST_{+1} are also highlighted using the corresponding letter from the single-letter code. Amino acids which are not identical but whose function is similar or comparable are marked by a :.

Fig. 7: Detail from a comparison of the protein sequences of the *Euplotes* p123 (p123), yeast (est2p) and human (phTC) catalytic telomerase subunits. The comparison, depicted in Fig. 5, between *Euplotes* p123 (p123), yeast (est2p) and humans (phTC) was carried out using the Clustal Method subprogram of the Lasergene program software (Dnastar, Inc.) under standard conditions. The amino acid residues are depicted in accordance with their single-letter code. The amino acids which are identical between yeast est2p, *Euplotes aediculatus* p123 and the identified EST_{+1} are also highlighted using the corresponding letter from the single-letter code. In addition, the regions which are identical between all three proteins are marked by a light grey bar above the protein sequence.

Fig. 8: Generated DNA sequence from Example 6 (RACE round 1) (SEQ ID No. 3).

Fig. 9: Generated DNA sequence from Example 6 (RACE round 2) (SEQ ID No. 4).

Fig. 10: Generated DNA sequence from Example 6 (RACE round 3) (SEQ ID No. 5).

Fig. 11: Generated DNA sequence from Example 8 (RACE round 3) (SEQ ID No. 6).

Fig. 12: Outline of the cloning of the complete hTC cDNA sequence. The positions of the start and stop codons are marked by arrows. The black regions of the rectangles symbolize protein-encoding sequence sections, whereas the pale grey regions symbolize 5'- and 3'- untranslated cDNA regions and/or denote intronsequences.

The dark grey blocks in the rectangle for the full-length cDNA either denote the telomerase-specific motif (T) or the seven reverse transcriptase motifs (numbers 1-7).

The DNA fragments which are required for preparing the complete hTC cDNA are likewise depicted as rectangles and are marked in accordance with their origin. All the rectangles are arranged in their positions relative to each other. The origin of the DNA fragment which is denoted by rectangle AA261296 is described in Example 2. The relative position of the 182 bp deletion in this fragment (compare Example 2) is shown by a gap in the rectangle. The origin of the DNA fragments which are denoted by the rectangles RACE 1, RACE 2 and RACE 3 is described in Example 6. The origin of the DNA fragment which is denoted by the C5F fragment rectangle is described in Example 7. The origin of the DNA fragment which is denoted by the lambda 12 rectangle is described in Example 9. The 3' part in the lambda 12 DNA fragment which encodes a cDNA which is not connected to hTC (compare Example 9) is not depicted in this figure. The complete hTC-cDNA sequence was joined together at the 5' and 3' splice sites using the lambda 12 and C5F DNA fragments shown in this figure (compare Example 7). These splice sites were identified in a variety of fragments (RACE 1, RACE 3, lambda 12 and C5F).

Fig. 13: Detailed sections from a comparison of the protein sequences of the catalytic telomerase subunits of *Euplotes* and man (hTC).

The figure shows sections from a comparison of the protein sequences of the catalytic telomerase subunits of *Euplotes* and man (hTC). Attention is drawn to the reverse transcriptase motifs in the boxed-in areas. The figures under the boxes refer to the respective amino acid positions in Fig. 2. The amino acid residues are shown in accordance with their single-letter code. Identical amino acids are printed in bold. In the consensus sequence for the reverse transcriptase (RT consensus) motif, h denotes a hydrophobic amino acid and p denotes a polar amino acid. If these groups of amino acids are retained in the *Euplotes* and hTC amino acid sequences, p and/or h is/are then printed in bold. Very highly conserved amino acids are underlaid in grey. In RT3, the boxed-in area is extended in order to cover

additional homologous amino acids. The telomerase-specific motif is described in Example 9.

Fig. 14: Generated DNA sequence from Example 11 (3' version) (SEQ ID No. 7). The region which is not homologous with the DNA sequence depicted in Fig. 1 is made to stand out in bold.

Fig. 15: hTC expression in cancer cell lines and normal human tissue. Fig. A: Approximately 2 μg of poly-A^+ RNA from different human cell lines were immobilized on the Northern blot in accordance with the manufacturer's (Clontech) instruction. Specifically, the RNA originated from a melanoma (G361), a lung carcinoma (A549), an adenocarcinoma of the colon (SW480), from a Raji Burkitt's lymphoma, from a leukaemia cell line (MOLT-4), from a chronic leukaemia cell line (K-562), from a cervical tumour (HeLa) and from the leukaemia cell line HL60. The transcripts marked 4.4 kb, 6 kb and 9.5 kb are specific for hTC (compare Example 10). Fig. B: About 2 μg of poly-A^+ RNA from different human tissues were immobilized on the Northern blot in accordance with the manufacturer's (Clontech) instructions. Specifically, the RNA was isolated from heart, brain, placenta, lung, liver, skeletal muscle, kidney and pancreas. An RNA size standard is shown.

Fig. 16: Western blot analysis of the rabbit sera against peptides from the human telomerase amino acid sequence (Example 12). In each case, 20 μl of the bacterial lysates from Example 13 were analysed in a western blot (Ausubel *et al.*, 1987) using the antisera from Example 12. Lysates from bacteria which harbour the pMALEST construct were loaded in lanes 1, 2, 6 and 7. Lysates from bacteria which harbour the pMALA1 construct were loaded in lanes 3, 4, 8 and 9. Lysates from bacteria which were not induced with IPTG (isopropyl-beta-thiogalactopyranoside) were loaded in lanes 1, 3, 6 and 8. Lysates from IPTG-induced bacteria were loaded in lanes 2, 4, 7 and 9. A standard size marker (10 kDa protein ladder from Life Technologies, Cat. No. 10064-012) was loaded

in lane 5. The 50 kDa and 120 kDa bands are marked at the edges of the membranes. The PVDF membrane in Fig. A containing lanes 1 to 4 was incubated with preimmune sera against peptide B (compare Example 12). The PVDF membrane in Fig. B containing lanes 6 to 9 was incubated with preimmune sera against peptide C (compare Example 12). The PVDF membrane in Fig. B containing lanes 1 to 4 was incubated with immune sera against peptide B (compare Example 12). The PVDF membrane in Fig. B containing lanes 6 to 9 was incubated with immune sera against peptide C (compare Example 12).

Fig. 17: Autoradiogram of ^{35}S-labelled, *in vitro*-translated protein. The complete *in vitro*-translated hTC (compare Example 15) was loaded in lane 1. A C-terminally truncated version of phTC was loaded in lane 2. Lane 3 shows a positive control for the *in vitro* translation which was supplied by the manufacturer (compare Example 15). A protein size standard for estimating protein sizes is marked on the right-hand side.

Fig. 18: Autoradiogram of ^{32}P-labelled products from the TRAP assay (compare Example 15). A TRAP assay mixture without any added enzyme or protein was loaded, as a negative control, in lanes 1 and 2. A TRAP assay mixture containing partially purified human telomerase from HeLa cells was loaded, as a positive control, in lanes 3 and 4. A TRAP assay mixture containing *in vitro*-translated phTC was loaded, undiluted, in lanes 5 and 6. A TRAP assay mixture containing *in vitro*-translated phTC, at a 1:4 dilution, was loaded in lanes 7 and 8. A TRAP assay mixture containing *in vitro*-translated phTC, at a dilution of 1:16, was loaded in lanes 9 and 10. A TRAP assay mixture containing *in vitro*-translated luciferase was loaded, as a negative control, in lanes 11 and 12.

Fig. 19: Autoradiogram of ^{32}P-labelled products from the direct telomerase assay (compare Example 15). A radioactively labelled 10 bp marker was loaded in lane 1. A telomer oligonucleotide ($[TTAGGG]_3$) which was radioactively labelled 5' was loaded in lane 2. Lane 3 is an empty lane. Partially purified human telomerase

from HeLa cells was used in a direct assay and the synthesis product was loaded, as a positive control, in lane 4. The *in vitro*-translated phTC from Example 15 was used in a direct assay and the synthesis product was loaded in lane 5.

Examples

Example 1

It is nowadays accepted that less than 5% of the human genome is in fact transcribed and translated into protein. Even before the genome has been completely sequenced, it is possible to obtain important information about the 60,000-70,000 genes in a human cell by investigating these coding moieties of the genome in a specific manner. The automation of high-throughput DNA sequencing technology in the last 10 to 15 years has made it possible to collect many cDNAs from plasmid cDNA libraries of widely differing origin and sequence the 5' or 3' end in each case. These short DNA sequences, which are typically of from 300 to 400 bp in length, are termed expressed sequence tags or ESTs for short and are compiled in various specialized data bases. The EST approach was initially described by Okubo *et al.* (1992) and transferred to a larger scale by Adams *et al.* (1992). At present, approximately 50,000 human cell genes are partially sequenced and documented as EST entries.

By comparing with the DNA and amino acid sequences of known genes, it is possible to identify related, but hitherto unkown, genes in these EST databases (Gerhold and Caskey, 1996). tBLASTn (Altschul *et al.*, 1990) is a search algorithm which has proved particularly useful for this purpose. This algorithm translates every DNA clone in the EST data base in all six possible reading frames and compares these amino acid sequences with the known protein sequence.

The EST data base at the National Center for Biotechnology Information (NCBI) was searched with the recently published protein sequence for the *Euplotes aediculatus* catalytic telomerase subunit *p123* (Lingner *et al.*, 1997). This resulted in a human EST with the accession number AA281296 being identified which exhibits significant homology with p123 in reading frame +1. This amino acid sequence in reading frame +1 is termed Est_{+1} in that which follows.

The homology between p123 and the Est_{+1} is most conspicuous in two sequence regions which are separated by 30 amino acids. The longer sequence region, which in p123 extends from amino acid 438 to amino acid 484, is 38% identical to the corresponding region Est_{+1}. If similar amino acids are also taken into consideration, the congruence is even 59%. The second block of homology extends, in the p123 protein, from amino acid 513 to amino acid 530 and exhibits 44% identity with the corresponding sequence segment in the identified Est_{+1}. A congruence of 61% is obtained when amino acid residues having similar properties are taken into account.

The P (probability) value is an important parameter for assessing a BLAST search. P indicates the probability of also finding a specific segment pair in a BLAST search using a random sequence and varies numerically between 0 (highly significant result) and 1 (insignificant result). Thus, comparison of the p123 equivalent from yeast (est2p) with the NCBI EST data base, for example, gave a negative result: The EST which was found had a probability of P=1 (Tab. 1). On the other hand, human telomerase-associated protein 1 (hTP1), which was found in an EST data base which is not available to the general public (Harrington *et al.*, 1997), gives a probability of P=0.004.

known gene (species)	**P**	**identified gene**	**origin of the cDNA library**
est2p (*Saccharomyces cerevisiae*	0.999	Rat EST	Kidney
p80 (*Tetrahymena termophilia*)	0.004	hTP1 (Harrington *et al.*, 1997)	Crypts of the intestinal epithelium
p123 (*Euplotes aediculatus*)	**$3.5^{x}10^{-06}$**	AA281296	Germinal centres of the tonsils

Tab. 1: Comparison of three tBlastn search runs using different known genes.

The human EST AA281296 which was identified by the comparison with p123 has a probability of $P=3.5\times10^{-6}$.

These data suggest that the identified EST in all probability encodes a fragment of the catalytic subunit of human telomerase. For this reason, the corresponding gene is abbreviated below to hTC (human Telomerase, catalytic) and the deduced protein is abbreviated to phTC.

Example 2

The EST which was identified by the comparison with p123 was fed into the EST data base on 2 April 1997 and has not been published in any journal. According to information obtained from the National Center for Biotechnology Information, the cDNA library which contains this EST clone was prepared as follows:

After the mRNA had been prepared from the germinal centres of the tonsils, a cDNA synthesis was carried out and the double-stranded cDNA fragments were cloned in an orientated manner, using the Not I and Eco RI restriction enzyme cleavage sites, into the vector pT7T3D-Pac.

The 389 bp which had been fed into the EST database were sequenced using the -28m13 rev2 primer supplied by Amersham (DNA sequence, see Fig. 1 Position 1685 to 2073).

Lasergene program software (Dnastar Inc.) was used to translate the DNA sequence of EST AA281296 in accordance with the human genetic code. The resulting amino acid sequence (Est_{+1}) corresponds to Position 542 to 670 in Fig. 2.

The deduced protein sequence of Est_{+1} is composed of 129 amino acids, including 27 basic, 11 acidic, 51 hydrophobic and 28 polar amino acid residues.

The EST (AA281296) which was identified in Example 1 was obtained commercially from Research Genetics, Inc. (Huntsville) in the form of a plasmid transformed into *E. coli* and analyse experimentally:

As shown in the ethidium bromide-stained agarose gel depicted in Fig. 3, a fragment from EST AA281296 of approximately 2.2 kb in size is liberated from the vector pT7T3D after subjecting the prepared plasmid DNA to restriction digestion. With the aid of a polymerase chain reaction (PCR), which was carried out in parallel and which made use of specific internal primers, EST AA281296 was inspected: the lengths of the expected PCR products are 325 and 380 bp and are in agreement with the lengths of the fragments which were found experimentally (cf. tracks 4 and 5 in Fig. 3). This therefore demonstrated that the E.coli clone supplied by Research Genetics, Int. (Huntsville) therefore harbours the identified EST as a plasmid.

After the DNA had been prepared, the 2176 bp of the insert in total were identified by means of double-strand sequencing. A comparison of the DNA sequences of clone AA281296 and of the C5F fragment (compare Example 7) showed that there was a 182 bp deletion (Positions 2352 to 2533, Fig. 1) and that the open reading frame is consequently displaced in this region. In summary, the DNA sequence of clone AA281296 is composed of the sequence information shown in Fig. 1 (Positions 1685 to 2351 and Positions 2534 to 4042).

Example 3

The tBLASTn comparison only identifies the regions in which there is the greatest agreement between p123 and Est_{+1} (amino acids 438-530, in p123), whereas the intervening amino acids are not taken into account. A Lipman-Pearson protein comparison was carried out in order to be able to draw conclusions about the relatedness of the protein sequences over a larger region (amino acids 437-554, in p123) (see Fig. 4). When this was done, 34% of the amino acids were found to be identical while 59% of the amino acids were found to be either identical or biochemically similar. This result demonstrates that the relatedness of these

proteins also continues outside the regions of homology which were found using the tBLASTn program.

As has recently been reported (Lingner *et al.*, 1997), *Euplotes aediculatus* p123 and *Saccharomyces cerevisiae* est2p are homologous to each other. In order to relate the degree of affinity between p123 and est2p to the homology between p123 and Est_{+1} which is described here, the Lipman-Pearson protein comparison was employed to compare the above-described region of p123 (amino acids 437-554) with est2p, too, using identical parameters. This showed that, in this chosen region, p123 and est2p are 21% identical and that 22% of their amino acid residues are either identical or biochemically similar (see Fig. 5). Accordingly, the homology between Est_{+1} and *Euplotes* p123 is significantly higher than between p123 and est2p.

Example 4

The homology of p123 with Est_{+1} and est2p suggests that all 3 proteins belong to the same protein family. In order to confirm this assumption, est2p was compared with Est_{+1} under the conditions described in Example 3 (see Fig. 6). This showed that Est_{+1} is 20% identical to est2p, that is exhibits a degree of homology which is comparable to that of p123 to est2p. This comparatively low level of congruence also confirms the finding that no significant EST was identified in the tBLASTn search using est2p (see Example 1).

Example 5

A computer comparison using p123, est2p and phTC was carried out in order to identify possibly functional domains which are important for the protein family consisting of catalytic telomerase subunits derived from different species (see Fig. 7). In this analysis, two regions which are present in all three proteins are particularly conspicuous (see Fig. 7). At present, no unambiguous function can be assigned to the region which, in p123, corresponds to amino acids 447 to 460 (Fig. 13, telomerase motif). A motif search using the Genetics Computer

Group (GCG) Wisconsin Sequence Analysis Package and a search in a protein data base (Swissprot, version of 8.6.1997) did not provide any significant insights.

On the other hand, a second region which is homologous between p123, est2p and phTC, corresponding in p123 to amino acids 512-526, exhibits a consensus motif for a reverse transcriptase (RT) (Figs. 7 and 13). Lingner *et al.*, (1997) showed that p123/est2p contain a total of 6 such RT motifs, which are essential for the catalytic function of p123/est2p. As depicted in Figs. 7 and 13, two such RT motifs are also conserved in the sequence of phTC which has been investigated. These motifs are the RT motifs which are located to the furthest extent N-terminally in p123/est2p (Lingner *et al.*, 1997).

The primary sequences of reverse transcriptases are strongly divergent; only a few amino acids are fully conserved within a separate motif (Poch *et al.*, 1989 and Xiong and Eickbush, 1990). In addition, due to having different distances between the conserved RT motifs, reverse transcriptases which are encoded by retroviruses or long terminal repeat (LTR) retroposons differ from those reverse transcriptases which are encoded by non-LTR retroposons or group II introns (Xiong and Eickbush, 1990). Based on the structure of their RT motifs, p123, est2p and phTC are to be assigned to the latter RT group. Interestingly, in this context, the consensus sequences of the RT motifs in phTC correspond most closely to the postulated RT consensus motif: of eight amino acid residues within the two RT motifs, 6 are present in the case of phTC while only 5 are present in the case of p123 and esp2p (Figs. 7 and 13). It is striking in this context how the hydrophobic amino acids, such as leucine and isoleucine, and the amino acids lysine and arginine, in particular, are in specific positions (Figs. 7 and 13).

In summary, it was hereby possible to demonstrate, at the descriptive level, that the AA281296 clone, identified due to its homology with p123, is a fragment of the catalytic subunit of human telomerase.

Example 6

For cloning the 5' end of the hTC-cDNA, three consecutive RACE (rapid amplification of cDNA ends) reactions were carried out in addition to the homology screening described in Example 8. Marathon-Ready cDNA (Clontech) form the human leukaemia cell line K562 or from human testis tissue was employed as the cDNA source. The implementation of the individual RACE rounds, as well as the results obtained, are described below.

In addition to this, the sequence information obtained in the RACE rounds was used in order to amplify the individual fragments from a contiguous cDNA clone by means of PCR.

RACE round 1:

In a final volume of 50 µl, 10 pmol of dNTP-mix were added to 5 µl of K562 Marathon-Ready cDNA (from Clontech, Catalogue Number 7441-1), and a PCR reaction was carried out in 1 × Klen Taq PCR reaction buffer and 1 × advantage Klen Taq polymerase mix (from Clontec). 10 pmol of the internal gene-specific primer GSP2 (5' -GCAACTTGCTCCAGACACTTCTTCCGG-3') from the 5' region of the hTC-EST clone and 10 pmol of the Marathon Adaptor primer AP1 (5'-CCATCCTAATACGACTCACTATAGGGC-3'; from Clontech) were added as primers. The PCR was carried out in 4 steps. After a one-minute denaturation at 94°C, denaturation was then carried out for 5 cycles of 30 sec at 94°C and the primers were then subsequently annealed for 4 min at 72°C and the DNA chain was extended. There then followed 5 cycles in which the DNA was denatured for 30 sec at 94°C but the subsequent primer extension took place for 4 min at 70°C. Finally, 22 cycles were then carried out in which, after the 30 sec DNA denaturation, the primer annealing and chain extension took place for 4 min at 68°C.

Following this PCR, the PCR product was diluted 1:50. 5 µl of this dilution were used in a second "nested" PCR together with 10 pmol of dNTP-mix in 1 × 10 Klen Taq PCR reaction buffer and 1 × Advantage Klen Taq polymerase mix and also 10 pmol of primer GSP2 and

10 pmol of the "nested" Marathon Adaptor primer AP2 (5'-ACTCACTATAGGGCTCGAGCGGC-3'; from Clontech). The PCR conditions corresponded to the parameters selected in the first PCR. As the only exception, only 16 cycles were chosen, instead of 22 cycles, in the last PCR step.

A DNA fragment of 1153 bp in length was obtained as the product of this nested RACE PCR. This fragment was cloned into the TA cloning vector pCR2.1 from Invitrogen and subjected to complete double-strand sequencing (Fig. 8 and SEQ ID No. 3).

Nucleotides 974 to 1153 represent the nucleotide region 1629 to 1808 of the hTC-cDNA which is depicted in Fig. 1. The nucleotide region extending from bp 1 to bp 973, which does not exhibit any homology with the hTC-cDNA sequence shown in Fig. 1, represents intron sequences of the hTC gene (data not shown). A 3' splice consensus sequence is located at the exon-intron transition. The presence of intron sequences could be due to using incompletely spliced mRNA as the starting substance for the cDNA synthesis. Genomic DNA contamination in the cDNA could also be an explanation for intron sequences being found.

RACE round 2:

Based on the sequence data obtained in the first RACE round, a second RACE was carried out using the gene-specific primer GSP5 from the 5' region of RACE product 1 (5'-GGCAGTGACCAGGAGGCAACGAGAGG-3') and the AP1 primer. Marathon-Ready cDNA from human testis (from Clontech; Catalogue Number 7414-1) was used as the cDNA source. The same PCR conditions were selected as in the 1st PCT in RACE round 1. The 1st PCR was also followed, in RACE round 2, by a 2nd "nested" PCR using diluted PCR product as the cDNA source. The gene-specific primer GSP6 from the 5' region of RACE product 1 (5'-GGCACACTCGGCAGGAAACGCACATGG-3') and the AP2 primer were used as the "nested" PCR primers. The conditions corresponded to parameters for the nested PCR from RACE round 1.

The PCR product of 412 bp in length from the nested PCR of RACE round 2 was cloned into the TA cloning vector pCRII-Topo from Invitrogen and sequence completely (Fig. 9 and SEQ ID No. 4). The sequence segment from bp 267 to bp 412 is completely homologous with the 5' region of the product from RACE 1. The region from bp 1 to bp 266 extends RACE product 1 at the 5' end. This RACE product 2 is probably, in its entirety, an intron region of the hTC gene (data not shown).

RACE round 3:

A third RACE round led to the identification of hTC-cDNA regions which were located further on in the 5' direction. Using the sequence results from RACE round 2 as a base, a gene-specific primer GSP9 (5'-CCTCCTCTGTTCACTGCTCTGGCC-3') was selected from the 5' region of RACE product 2 and used in a new RACE together with the AP1 primer and Marathon-Ready cDNA from human testis (from Clontech). The RACE conditions were the same as those used in the 1st PCR in RACEs 1 and 2. In the "nested" RACE which followed, and which took place, in accordance with the "nested" RACEs in rounds 1 and 2, using the gene-specific primer GSP10 from the 5' region of RACE product 2 (5'-CGTAAGTTTATGCAAACTGGACAGG-3') and AP2, a fragment of 1012 bp in length (Fig. 10 and SEQ ID No. 5) was amplified and cloned into the TA cloning vector pCRII--TOPO. Subsequent sequencing showed that the 3' region of this RACE fragment (bp 817 - bp 1012) evidently still constitutes an intron sequence of the hTC gene. The region from bp 889 to bp 1012 is completely homologous with the 5' region of RACE product 2. On the other hand, the 5' region of this fragment, from bp 1 to bp 816, is identical to the bp 814 - bp 1629 region of the hTC-cDNA which is shown in Fig. 1. A potential 5' splice consensus sequence is located at the exon-intron transition.

Example 7

A PCR was carried out in order to clone a contiguous fragment from the sequence information obtained from RACE 2 and clone AA281296. Marathon-Ready cDNA from human testis (from Clontech; Catalogue Number 7414-1) was used as the cDNA source. The

PCR mixture was as described under RACE 1 (compare Example 6) but using the primers C5F (5'-CGAGTGGACACGGTGATCTCTGCC-3') from the 5' region of RACE 2 and primer C3B (5'-GCACACCTTTGGTCACTCCAAATTCC-3') from a 3' region of clone AA281296. The PCR was carried out in 2 steps. After a one-minute denaturation at 94°C, denaturation was then carried out for 36 cycles of 30 sec at 94°C and, after that, the primers were annealed, and the DNA chain was extended, for 4 min at 68°C.

A DNA fragment of 2486 bp in length, which is designated the C5F fragment below, was obtained as the product of this PCR. This fragment was cloned into the TA cloning vector pCRII-TOPO from Invitrogen and subjected to complete double-strand sequencing. A comparison of the DNA sequences of the C5F fragment and the AA281296 clone showed that there was an in-frame insertion of 182 bp between RT motif 3 and RT motif 4 (Positions 2352 to 2533, Fig. 1). A further comparison of DNA of the C5F fragment with the sequences from the three RACE rounds made it clear that an intron which was already identified in RACE 2 was present at the 3' end of C5F. A 3' splice consensus sequence is located at the exon-intron transition. In summary, the DNA sequence of the C5F fragment is consequently composed of the sequence information shown in Fig. 9 (Position 64 to 278) and the sequence data shown in Fig. 1 (Positions 1636 to 3908).

Example 8

For cloning the 5' end of the hTC-cDNA, a homology screening (Ausubel *et al.*, 1987) was carried out in addition to the RACE protocol described in Example 6. A human erythroleukaemia 5'-stretch plus cDNA library (from Clontech, cat. No. HL5016b) from the human leukaemia cell line K562 was used as the cDNA source. Approximately 3×10^6 Pfu of this random and oligo-dT-primed library were plated out and used for screening as described in Ausubel *et al.* (1987). A radioactively labelled hTC-DNA fragment of 719 bp in length (Positions 1685 to 2404, corresponding to Fig. 1) was used as the probe.

Following a rescreening with the same hTC probe, the λ clone 12 was verified as being positive out of 20 putatively positive λ clones. Following plaque purification and λ DNA

preparation (Ausubel *et al.*, 1987), the 4 kb insert was recloned into the pBluescript vector and sequenced (Fig. 11 and SEQ ID No. 6).

A comparison of the λ clone 12 sequence with the sequences of the RACE clones and the DNA sequence of clone AA281296 showed that this clone, which was identified in the homology screening, encodes a 5' part of the hTC-cDNA and possesses a putative ATG start codon in Position 63 in accordance with Fig. 1. There is no stop codon in the same reading frame 5' of this ATG. Subsequent sequence analyses make it clear that λ clone 12 probably contains an intron from Positions 1656 to 2004. Very well conserved 5' and 3' splice sites provide support for this hypothesis. The hTC-cDNA-encoding sequence then continues from Position 2005 to Position 2382. The sequence from 2383 to the 3' end of λ clone 12 exhibits a conspicuous open reading frame in reading frame -4. A bioinformatic analysis of the corresponding DNA sequence showed that, over about 400 bp, this reading frame is identical to a variety of ESTs which have no connection with the hTC cDNA. Consequently, λ clone 12 is a chimeric clone which essentially consists of the 5' end of the hTC cDNA and another cDNA clone of unknown function.

A diagrammatic summary showing the relative orientations of the RACE products, and the homology screening, is depicted in Fig.12. The complete sequence of the hTC cDNA (Fig. 1) was assembled from λ clone 12 (Positions 21 to 1655 in accordance with Fig. 11), the C5F PCR product (Positions 1636 to 3908 in accordance with Fig. 1) and EST AA281296 (Positions 3909 to 4042, in accordance with Fig. 1).

Example 9

A total of seven motifs for reverse transcriptases (RT motifs) was identified by comparing the phTG protein sequence (Fig. 2 and SEQ ID NO. 2) with a reverse transcriptase consensus sequence (Poch *et al.*, 1989, Xiong and Eickbush, 1990) (Fig. 13). Within these motifs, some amino acids are highly conserved not only between the RT consensus sequence and phTC but also in comparison with the *Euplotes* telomerase protein. Thus, two aspartic acids (Positions 868 and 869 in Fig. 2) are, for example, completely conserved in RT motif 5 (Fig. 13). RT

motif 7, which was deduced from other reverse transcriptases (Poch *et al.*, 1989, Xiong and Eickbush, 1990), was only demonstrated in the human catalytic telomerase subunit and not in the *Euplotes* protein (Fig. 13).

Structural features which can only be found in the telomerase proteins and not in other reverse transcriptases are also conspicuous. The telomerase motif (Positions 553 and 565 in Fig. 2) is a structure which is specific for this protein family since it does not occur in any previously known protein. A further feature which has only been identified in the catalytic telomerase proteins is the difference between RT motifs 3 and 4, which distance, at 107 amino acids, is markedly greater than in other RTs. These special features indicate that the catalytic subunits of the telomerases from different species probably constitute a separate subgroup of RNA-dependent DNA polymerases.

Example 10

Expression of the telomerase RNA subunit (hTR) does not correlate with telomerase activity but, instead, is observed ubiquitously (Feng *et al.*, 1995). Consequently, the question arises as to whether expression of the catalytic telomerase subunit is associated with telomerase activity.

Northern blot experiments (Ausubel *et al.*, 1987) were carried out in order to analyze the level of hTC expression. The commercially available Northern blots were supplied with a number of RNA preparations from normal human tissue (from Clontech; catalogue No. 7760-1) or with RNA samples from human cancer cell lines (from Clontech; Catalogue Number 7757-1). A radioactively labelled hTC DNA fragment of 719 bp in length (Positions 1685 to 2404, in accordance with Fig. 1) was used as the probe. The membranes were incubated with the probe in accordance with the manufacturer's (Clontech) instructions.

Two main RNA transcripts, of about 9.5 kb and 4.4 kb in size, and an additional RNA transcript of about 6 kb, which transcripts cross-hybridize with the probe, were detected in the eight human cell lines (3 leukaemia cell lines, 3 carcinoma cell lines, one melanoma and one

lymphoma) tested (Fig.15, Fig. A). In the comparison, the hTC mRNA was expressed most strongly in the leukaemia cell lines K-562 and HL-60 (Fig. 15, Fig. A). By contrast, it was not possible to detect the hTC transcript in the normal tissues (heart, brain, placenta, lung, liver, skeletal muscle, kidney and pancreas) which were tested (Fig. 15, Fig. B). This observation is not surprising since it was not possible to detect any telomerase activity, either, in these tissues (Kim *et al.*, 1994).

These data indicate that the induction of hTC expression plays an important role in activating the telomerase during tumour development.

Example 11

Several PCR products, whose sizes only differed from each other to a minimal extent, were always obtained when the hTC cDNA fragments from various cDNA libraries (Clontech Marathon Ready cDNA from the human leukaemia cell line K562 and from human testis and also cDNA from the human premyeloid leukaemia cell line HL60) were subjected to PCR amplification. In order to elucidate the differences between the different hTC-PCR products, a fragment of the hTC cDNA depicted in Fig. 1 extending from bp 1783 to bp 3901 was amplified using the primers C5A (5'-CCGGAAGAGTGTCTGGAGCAAGTTGC-3') and C3B (5'-GCACACCTTTGGTCACTCCAAATTCC-3'). Marathon-Ready cDNA from K562 leukaemia cells (from Clontech; Catalogue Number 7441-1) was used as the cDNA source (PCR1 and 2). In a third PCR, a hTC fragment, from bp 1695 to bp 3463, of the hTC cDNA in Fig. 1 was amplified from HL60 cDNA using the primers GSP1 front (5'-GGCTGATGAGTGTGTACGTCGTCGAG-3') and HTRT3A (5'-GGGTGGCCATCAGTCCAGGATGG-3').

The conditions of the 3 PCR reactions are described below:

In the first PCR, and in a final volume of 50 µl, 10 pmol of dNTP mix were added to 5 µl of K562 Marathon-Ready cDNA, and a PCR reaction was carried out in 1 × Klen Taq PCR reaction buffer and 1 × Advantage Klen Taq polymerase mix (from Clontech). 10 pmol of

each of the primers C5A and C5B were added. The PCR was carried out in 3 steps. A one-minute denaturation at 94°C was followed by 35 PCR cycles in which the DNA was firstly denatured for 30 sec at 94°C and the primers were then annealed, and the DNA chain was extended, for 4 min at 68°C. In conclusion, there followed a chain extension for 10 min at 68°C. The resulting PCR products were cloned into the TA cloning vector pCRII-TOPO from Invitrogen.

In a second PCR, 10 pmol of each of the primers C5A and C3B, 10 pmol of dNTP mix and 2 U of Taq DNA polymerase (from Gibco-BRL) were added to 5 µl of K562 Marathon-Ready cDNA, and a PCR reaction was carried out in 1 × PCR buffer (from Perkin Elmer) in a final volume of 50 µl. The PCR reaction was carried out in 3 steps. The DNA was firstly denatured for 3 min at 94°C. There then followed 34 cycles in which, consecutively, the DNA was denatured for 45 sec at 94°C, primer annealing then took place for 1 min at 68°C and, after that, the DNA chain was extended for 3 min at 72°C. In the last PCR step, a concluding chain extension was carried out for 10 min at 72°C. The resulting PCR products were cloned into the TA cloning vector pCR2.1 from Invitrogen.

For the third PCR, the cDNA synthesis kit from Boehringer Mannheim was first of all used to carry out a cDNA synthesis from 2 µg of DNaseI-treated poly-A RNA from the human premyeloid cell line HL60 in accordance with the manufacturer's instructions. 1 µl of this HL60 cDNA was then mixed with 10 pmol of each of the primers GSP1 front and HTRT3A and also 10 pmol of dNTP mix, in a final volume of 50 µl, and, after 1.25 µl of DMSO in 1 × Klen Taq PCR reaction buffer and 1 × Advantage Klen Taq polymerase mix (from Clontech) had been added, a PCR reaction was carried out. The PCR reaction proceeded in 3 steps. After a denaturation for 3 min at 94°C, the DNA was initially denatured for 1 min at 94°C and the primers were then annealed, and the DNA chain extended, for 4 min at 68°C, over 37 cycles. The reaction was concluded by a further incubation for 10 min at 68°C. The PCR products were cloned into the TA cloning vector pCR2.1-TOPO.

Complete double-strand sequencing of the cloned hTC cDNA fragments from PCRs 1 and 2, and partial sequencing of the hTC cDNA fragments obtained from PCR 3, showed that, in addition to the hTC cDNA depicted in Fig. 1, 4 variants of this cDNA exist in human cells, i.e.:

Variant 1 of human hTC cDNA is distinguished by a deletion of 182 bp in length extending from nucleotides 2345 to 2526. This deletion results in the ORF being displaced, with a truncated hTC protein, which lacks RT motifs 4 to 7, being read off.

Variant 2 of human hTC cDNA exhibits a deletion of 36 bp in length extending from nucleotides 2184 to 2219. RT motif 3 is lost as a result of this deletion. However, the reading frame is retained and a protein is produced which selectively lacks RT motif 3.

Variant 3 of human hTC cDNA is a combination of variants 1 and 2. It exhibits both a deletion from bp 2184 to 2219 and a deletion from bp 2345 to 2526.

Variant 4 of human hTC cDNA is distinguished by the loss of the nucleotide region from bp 3219 to bp 3842. This missing sequence is replaced by a sequence which is not homologous with hTC. From bp 3843 onwards, the sequence is once again completely identical to the hTC sequence depicted in Fig. 1. The sequence of variant 4 is shown in Fig. 14. In accordance with the 5' primer chosen, it begins with bp 1783 of the hTC cDNA shown in Fig. 1. The region which is not homologous is emphasized in bold and, from Position 3219 to Position 3451 (Fig. 14 and SEQ ID No. 7) is, to the extent of 98.7%, in agreement, at the DNA level, with an EST (Accession No. AA299878) from a human uterus tumour.

Exanple 12

In order to obtain antisera having specificity for the catalytic subunit of human telomerase, the available nucleotide sequence (Fig. 1) was translated into an amino acid sequence (Fig. 2). Using a secondary structure prediction program (PROTEAN, from the DNAStar

software package, DNASTAR Inc., Madison, WI, USA), two peptides were chosen which, with a certain degree of probability, evoke an immune response. These are the following peptides, which are depicted in the one-letter code for amino acids:

B: C-K-R-V-Q-L-R-E-L-S-E-A-E-V-R-Q - $CONH_2$/Pos. 594 - 608

C: C-Q-E-T-S-P-L-R-D-A-V-V-I-E-Q-S-S-S-L-N-E - $CONH_2$/Pos. 781-800

The cysteines which are underlined are not derived from the telomerase sequence but were additionally added on as linkers for the coupling.

The peptides were coupled to keyhole limpet hemocyanin (KLH) using the thiol-reactive coupling reagent m-maleimidobenzoyl-N-hydroxysuccinimide ester (MBS). Two rabbits were in each case immunized with these coupled peptides at intervals of from 2 to 4 weeks. Prior to immunization, 5 ml of blood were withdrawn in order to obtain preimmune sera. After 4 immunizations, 5 ml of blood were likewise withdrawn for obtaining immune sera. These sera were tested for reactivity with fusion proteins (Example 13) in a Western blotting experiment (Ausubel *et al.*, 1987).

Example 13

Bacterial expression experiments were carried out in order to be able to analyse the protein of the catalytic telomerase subunit.

The constructs of these experiments are described below:

For the expression construct pMalEST, the insert in the AA281296 clone mentioned in Example 2 was excised with restriction enzymes Eco RI and Not I and the cleavage sites were filled in using the Klenow fragment (Ausubel *et al.*, 1987); the insert was then cloned into the given reading frame of the maltose-binding protein of the bacterial expression vector pMAL-C2 (from New England Biolabs). Vector pMAL-C2 was digested with restriction

enzyme Pst I and the protruding single-strand ends were removed with T4 DNA polymerase (Ausubel *et al.*, 1987).

The expression construct pMalA1 contains the nucleotide sequence of Fig. 1 from Position 1789 to Position 3908. This DNA fragment was amplified from a commercially available K562 Marathon-Ready cDNA library (from Clontech, Catalogue Number 7441-1) by means of PCR using the primers C5A (5'-ACCGGAAGAGTGTCTGGAGCAAGTTG-3') and C3B (5'-GCACACCTTTGGTCACTCCAAATTCC-3'), and cloned into the TA cloning vector pCRII-TOPO from Invitrogen. The PCR conditions were as described in Example 7. For the expression construct pMalA1, the insert was excised using the restriction enzyme Eco RI and the cleavage sites were filled in using the Klenow fragment (Ausubel *et al.*, 1987); the insert was then cloned into the bacterial expression vector pMAL-C2 (from New England Biolabs) which had been cleaved with the restriction enzyme Xmn I.

These constructs were then used for protein expression in the bacterial strain *E. coli* DH5α. The expression conditions were those as described in the instructions provided by New England Biolabs (Catalogue Number 800). The bacterial lysates which were prepared were tested in a Western blotting experiment (Ausubel *et al.*, 1987).

Example 14

The bacterial lysates from Example 13 were analysed in a Western blot (Ausubel *et al.*, 1987) using the antisera from Example 12.

Since the proportion of the fusion represented by the maltose-binding protein is about 43 kDa in size, fusion proteins of about 74 kDa and 106 kDa are expected for the pMalEST and pMalA1 constructs, respectively.

When comparing the preimmune sera with the sera following the first immunization, it becomes evident that specific antibodies were formed against the B and C epitopes (Fig. 16). Furthermore, in addition to the expected 74 kDa and 106 kDa proteins, respectively, smaller

protein fragments were also observed which react with the antisera. These smaller products probably originate from premature products.

Only the epitope for serum B is present on the fusion protein from the expression using pMalEST. By contrast, the epitopes for sera B and C are present on the fusion protein from pMalA1. For this reason, antiserum C does not recognize the pMalEST expression product and only recognizes the larger protein fragments from the expression experiments using pMalA1. This observation underlines the high degree of specificity of the antisera which were generated.

Example 15

In order to be able to analyse the protein of the catalytic telomerase subunit, the protein component should be reconstituted *in vitro* together with the RNA component.

The constructs for these experiments are described below:

The RNA component of 504 nt in length (Feng *et al.*, 1995) was amplified from a 293 cell cDNA library using the primers HTR9BAM (5'-CGCGG-ATCCTAATACGACTCACTATAGGGTTGCGGAGGGTGGGCCTG-3') and HTR2BAM (5'-CGCGGATCCCGGCGAGGGGTGACGGATGC-3). Primer HTR9BAM contains a T7 promoter from nucleotide 10 to 29. In the PCR, 10 pmol of dNTP mix were added, in a final volume of 100 µl, to 3 µl of cDNA from 293 cells, and a PCR reaction was carried out in 1 × PCR reaction buffer containing 0.5 µl of Taq polymerase (from Gibco). 10 pmol of each of the primers HTR9BAM and HTR2BAM were added. The PCR was carried out in 3 steps. A ten-minute denaturation at 94°C was followed by 35 PCR cycles in which the DNA was first of all denatured for one minute at 94°C and, after that, the primers were annealed, and the DNA chain was extended, for 2 min at 62°C. In conclusion, there followed a chain extension for 4 min at 72°C. The resulting PCR products were cloned, after a restriction digestion with Bam HI, into the Bam HI cleavagē site of vector pUC19 in such a way that the

RNA component is under the control of the T7 promoter. This construct is designated HTR504 in that which follows.

The cDNA fragment of 3411 bp in length (Position 60 to Position 3470, Fig. 1) was cloned into the vector PCRII TOPO (from Invitrogen). Detailed information on the cloning is given in Examples 8 and 7, and also in Fig. 12. In this construct, which is designated HTC FL, the T7 promoter is located 5' before the hTC cDNA.

The catalytic telomerase protein component was synthesized in a commercially available transcription/translation system, after adding the hTC FL construct, in accordance with the manufacturer's (Promega; Catalogue Number L4610) instructions. Whether the *in vitro* translation of the expected 127 kDa product had been successful was checked in an SDS-PAGE (Ausubel *et al.*, 1987) using ^{35}S-labelled cysteine (Fig. 17).

The telomerase RNA component was synthesized using a transcription system in accordance with the manufacturer's (Ambion; Catalogue Number 1344) instructions or using the method described by Pokrovskaya and Gurevich (1994).

For the *in vitro* re-constitution, 0.5 μg of hTRNA was added to 50 μl of the above-described translation mixture containing the hTC FL construct and the whole was incubated at 37°C for 10 min. The enzymatic activity of 2 μl of this mixture was investigated using the TRAP assay (N.W. Kim *et al.*, 1994). The measurement of the activity, by the same method, of telomerase which was purified from HeLa cells (Shay *et al.*, 1994) was used as the positive control. As can be seen in Fig. 18, both the reconstituted enzyme and the native enzyme produce the same product pattern, i.e. the nucleotide ladder which is characteristic for telomerase. This result also verifies that a single protein component, together with the RNA, is sufficient for the enzymatic telomerase activity.

In addition to the described TRAP assay, 5 μl of the reconstitution mixture were tested for its activity in a direct telomerase assay (Shay *et al.*, 1994). In this experiment, too, the

characteristic nucleotide ladder verifies the successful reconstitution of recombinant hTC protein and telomerase RNA component.

In summary, it was hereby possible to demonstrate, at the functional level, that the identified, and completely cloned, hTC-cDNA constitutes the catalytic subunit of human telomerase.

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SEQUENCE LISTING

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(iii) NUMBER OF SEQUENCES: 7

(iv) COMPUTER-READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30B (EPA)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4042 Basenpaare
(B) TYPE: Nucleotide
(C) STRANDEDNESS: Einzelstrang
(D) TOPOLOGY: Linear

(ii) ART DES MOLEKšLS: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(vi) ORIGINAL SOURCE:
(C) INDIVIDUAL/ISOLATE: Human

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

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TTCAGATGCC GGCCCACGGC CTATTCCCCT GGTGCGGCCT GCTGCTGGAT ACCCGGACCC 2880

TGGAGGTGCA GAGCGACTAC TCCAGCTATG CCCGGACCTC CATCAGAGCC AGTCTCACCT 2940

TCAACCGCGG CTTCAAGGCT GGGAGGAACA TGCGTCGCAA ACTCTTTGGG GTCTTGCGGC 3000

TGAAGTGTCA CAGCCTGTTT CTGGATTTGC AGGTGAACAG CCTCCAGACG GTGTGCACCA 3060

ACATCTACAA GATCCTCCTG CTGCAGGCGT ACAGGTTTCA CGCATGTGTG CTGCAGCTCC 3120

CATTTCATCA GCAAGTTTGG AAGAACCCCA CATTTTTCCT GCGCGTCATC TCTGACACGG 3180

CCTCCCTCTG CTACTCCATC CTGAAAGCCA AGAACGCAGG GATGTCGCTG GGGGCCAAGG 3240

GCGCCGCCGG CCCTCTGCCC TCCGAGGCCG TGCAGTGGCT GTGCCACCAA GCATTCCTGC 3300

TCAAGCTGAC TCGACACCGT GTCACCTACG TGCCACTCCT GGGGTCACTC AGGACAGCCC 3360

AGACGCAGCT GAGTCGGAAG CTCCCGGGGA CGACGCTGAC TGCCCTGGAG GCCGCAGCCA 3420

ACCCGGCACT GCCCTCAGAC TTCAAGACCA TCCTGGACTG ATGGCCACCC GCCCACAGCC 3480

AGGCCGAGAG CAGACACCAG CAGCCCTGTC ACGCCGGGCT CTACGTCCCA GGGAGGGAGG 3540

GGCGGCCCAC ACCCAGGCCC GCACCGCTGG GAGTCTGAGG CCTGAGTGAG TGTTTGGCCG 3600

AGGCCTGCAT GTCCGGCTGA AGGCTGAGTG TCCGGCTGAG GCCTGAGCGA GTGTCCAGCC 3660

AAGGGCTGAG TGTCCAGCAC ACCTGCCGTC TTCACTTCCC CACAGGCTGG CGCTCGGCTC 3720

CACCCCAGGG CCAGCTTTTC CTCACCAGGA GCCCGGCTTC CACTCCCCAC ATAGGAATAG 3780

TCCATCCCCA GATTCGCCAT TGTTCACCCC TCGCCCTGCC CTCCTTTGCC TTCCACCCCC 3840

ACCATCCAGG TGGAGACCCT GAGAAGGACC CTGGGAGCTC TGGGAATTTG GAGTGACCAA 3900

AGGTGTGCCC TGTACACAGG CGAGGACCCT GCACCTGGAT GGGGGTCCCT GTGGGTCAAA 3960

TTGGGGGGAG GTGCTGTGGG AGTAAAATAC TGAATATATG AGTTTTTCAG TTTTGAAAAA 4020

AAAAAAAAAA AAAAAAAAAA AA 4042

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1132 amino acids
(B) TYPE: Amino acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(ii) ART DES MOLEKšLS: Protein

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(vi) ORIGINAL SOURCE:
(C) INDIVIDUAL/ISOLATE: Human

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

```
Met Pro Arg Ala Pro Arg Cys Arg Ala Val Arg Ser Leu Leu Arg Ser
1               5                   10                  15

His Tyr Arg Glu Val Leu Pro Leu Ala Thr Phe Val Arg Arg Leu Gly
            20                  25                  30

Pro Gln Gly Trp Arg Leu Val Gln Arg Gly Asp Pro Ala Ala Phe Arg
        35                  40                  45

Ala Leu Val Ala Gln Cys Leu Val Cys Val Pro Trp Asp Ala Arg Pro
    50                  55                  60

Pro Pro Ala Ala Pro Ser Phe Arg Gln Val Ser Cys Leu Lys Glu Leu
65                  70                  75                  80

Val Ala Arg Val Leu Gln Arg Leu Cys Glu Arg Gly Ala Lys Asn Val
                85                  90                  95

Leu Ala Phe Gly Phe Ala Leu Leu Asp Gly Ala Arg Gly Gly Pro Pro
            100                 105                 110

Glu Ala Phe Thr Thr Ser Val Arg Ser Tyr Leu Pro Asn Thr Val Thr
        115                 120                 125

Asp Ala Leu Arg Gly Ser Gly Ala Trp Gly Leu Leu Leu Arg Arg Val
    130                 135                 140

Gly Asp Asp Val Leu Val His Leu Leu Ala Arg Cys Ala Leu Phe Val
145                 150                 155                 160

Leu Val Ala Pro Ser Cys Ala Tyr Gln Val Cys Gly Pro Pro Leu Tyr
                165                 170                 175

Gln Leu Gly Ala Ala Thr Gln Ala Arg Pro Pro Pro His Ala Ser Gly
            180                 185                 190

Pro Arg Arg Arg Leu Gly Cys Glu Arg Ala Trp Asn His Ser Val Arg
        195                 200                 205

Glu Ala Gly Val Pro Leu Gly Leu Pro Ala Pro Gly Ala Arg Arg Arg
    210                 215                 220

Gly Gly Ser Ala Ser Arg Ser Leu Pro Leu Pro Lys Arg Pro Arg Arg
225                 230                 235                 240
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Gly Ala Ala Pro Glu Pro Glu Arg Thr Pro Val Gly Gln Gly Ser Trp
                245                 250                 255

Ala His Pro Gly Arg Thr Arg Gly Pro Ser Asp Arg Gly Phe Cys Val
            260                 265                 270

Val Ser Pro Ala Arg Pro Ala Glu Glu Ala Thr Ser Leu Glu Gly Ala
        275                 280                 285

Leu Ser Gly Thr Arg His Ser His Pro Ser Val Gly Arg Gln His His
    290                 295                 300

Ala Gly Pro Pro Ser Thr Ser Arg Pro Pro Arg Pro Trp Asp Thr Pro
305                 310                 315                 320

Cys Pro Pro Val Tyr Ala Glu Thr Lys His Phe Leu Tyr Ser Ser Gly
                325                 330                 335

Asp Lys Glu Gln Leu Arg Pro Ser Phe Leu Leu Ser Ser Leu Arg Pro
            340                 345                 350

Ser Leu Thr Gly Ala Arg Arg Leu Val Glu Thr Ile Phe Leu Gly Ser
        355                 360                 365

Arg Pro Trp Met Pro Gly Thr Pro Arg Arg Leu Pro Arg Leu Pro Gln
    370                 375                 380

Arg Tyr Trp Gln Met Arg Pro Leu Phe Leu Glu Leu Leu Gly Asn His
385                 390                 395                 400

Ala Gln Cys Pro Tyr Gly Val Leu Leu Lys Thr His Cys Pro Leu Arg
                405                 410                 415

Ala Ala Val Thr Pro Ala Ala Gly Val Cys Ala Arg Glu Lys Pro Gln
            420                 425                 430

Gly Ser Val Ala Ala Pro Glu Glu Glu Asp Thr Asp Pro Arg Arg Leu
        435                 440                 445

Val Gln Leu Leu Arg Gln His Ser Ser Pro Trp Gln Val Tyr Gly Phe
    450                 455                 460

Val Arg Ala Cys Leu Arg Arg Leu Val Pro Pro Gly Leu Trp Gly Ser
465                 470                 475                 480

Arg His Asn Glu Arg Arg Phe Leu Arg Asn Thr Lys Lys Phe Ile Ser
                485                 490                 495

Leu Gly Lys His Ala Lys Leu Ser Leu Gln Glu Leu Thr Trp Lys Met
            500                 505                 510

Ser Val Arg Asp Cys Ala Trp Leu Arg Arg Ser Pro Gly Val Gly Cys
        515                 520                 525

Val Pro Ala Ala Glu His Arg Leu Arg Glu Glu Ile Leu Ala Lys Phe
    530                 535                 540

Leu His Trp Leu Met Ser Val Tyr Val Val Glu Leu Leu Arg Ser Phe
545                 550                 555                 560

Phe Tyr Val Thr Glu Thr Thr Phe Gln Lys Asn Arg Leu Phe Phe Tyr
                565                 570                 575
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Arg Lys Ser Val Trp Ser Lys Leu Gln Ser Ile Gly Ile Arg Gln His
            580                 585                 590

Leu Lys Arg Val Gln Leu Arg Glu Leu Ser Glu Ala Glu Val Arg Gln
        595                 600                 605

His Arg Glu Ala Arg Pro Ala Leu Leu Thr Ser Arg Leu Arg Phe Ile
    610                 615                 620

Pro Lys Pro Asp Gly Leu Arg Pro Ile Val Asn Met Asp Tyr Val Val
625                 630                 635                 640

Gly Ala Arg Thr Phe Arg Arg Glu Lys Arg Ala Glu Arg Leu Thr Ser
                645                 650                 655

Arg Val Lys Ala Leu Phe Ser Val Leu Asn Tyr Glu Arg Ala Arg Arg
            660                 665                 670

Pro Gly Leu Leu Gly Ala Ser Val Leu Gly Leu Asp Asp Ile His Arg
        675                 680                 685

Ala Trp Arg Thr Phe Val Leu Arg Val Arg Ala Gln Asp Pro Pro Pro
    690                 695                 700

Glu Leu Tyr Phe Val Lys Val Asp Val Thr Gly Ala Tyr Asp Thr Ile
705                 710                 715                 720

Pro Gln Asp Arg Leu Thr Glu Val Ile Ala Ser Ile Ile Lys Pro Gln
                725                 730                 735

Asn Thr Tyr Cys Val Arg Arg Tyr Ala Val Val Gln Lys Ala Ala His
            740                 745                 750

Gly His Val Arg Lys Ala Phe Lys Ser His Val Ser Thr Leu Thr Asp
        755                 760                 765

Leu Gln Pro Tyr Met Arg Gln Phe Val Ala His Leu Gln Glu Thr Ser
    770                 775                 780

Pro Leu Arg Asp Ala Val Val Ile Glu Gln Ser Ser Ser Leu Asn Glu
785                 790                 795                 800

Ala Ser Ser Gly Leu Phe Asp Val Phe Leu Arg Phe Met Cys His His
                805                 810                 815

Ala Val Arg Ile Arg Gly Lys Ser Tyr Val Gln Cys Gln Gly Ile Pro
            820                 825                 830

Gln Gly Ser Ile Leu Ser Thr Leu Leu Cys Ser Leu Cys Tyr Gly Asp
        835                 840                 845

Met Glu Asn Lys Leu Phe Ala Gly Ile Arg Arg Asp Gly Leu Leu Leu
    850                 855                 860

Arg Leu Val Asp Asp Phe Leu Leu Val Thr Pro His Leu Thr His Ala
865                 870                 875                 880

Lys Thr Phe Leu Arg Thr Leu Val Arg Gly Val Pro Glu Tyr Gly Cys
                885                 890                 895

Val Val Asn Leu Arg Lys Thr Val Val Asn Phe Pro Val Glu Asp Glu
```

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            900                 905                 910

Ala Leu Gly Gly Thr Ala Phe Val Gln Met Pro Ala His Gly Leu Phe
        915                 920                 925

Pro Trp Cys Gly Leu Leu Leu Asp Thr Arg Thr Leu Glu Val Gln Ser
    930                 935                 940

Asp Tyr Ser Ser Tyr Ala Arg Thr Ser Ile Arg Ala Ser Leu Thr Phe
945                 950                 955                 960

Asn Arg Gly Phe Lys Ala Gly Arg Asn Met Arg Arg Lys Leu Phe Gly
                965                 970                 975

Val Leu Arg Leu Lys Cys His Ser Leu Phe Leu Asp Leu Gln Val Asn
            980                 985                 990

Ser Leu Gln Thr Val Cys Thr Asn Ile Tyr Lys Ile Leu Leu Leu Gln
        995                 1000                1005

Ala Tyr Arg Phe His Ala Cys Val Leu Gln Leu Pro Phe His Gln Gln
    1010                1015                1020

Val Trp Lys Asn Pro Thr Phe Phe Leu Arg Val Ile Ser Asp Thr Ala
1025                1030                1035                1040

Ser Leu Cys Tyr Ser Ile Leu Lys Ala Lys Asn Ala Gly Met Ser Leu
                1045                1050                1055

Gly Ala Lys Gly Ala Ala Gly Pro Leu Pro Ser Glu Ala Val Gln Trp
            1060                1065                1070

Leu Cys His Gln Ala Phe Leu Leu Lys Leu Thr Arg His Arg Val Thr
        1075                1080                1085

Tyr Val Pro Leu Leu Gly Ser Leu Arg Thr Ala Gln Thr Gln Leu Ser
    1090                1095                1100

Arg Lys Leu Pro Gly Thr Thr Leu Thr Ala Leu Glu Ala Ala Ala Asn
1105                1110                1115                1120

Pro Ala Leu Pro Ser Asp Phe Lys Thr Ile Leu Asp
                1125                1130
```

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 1153 base pairs
- (B) TYPE: Nucleotide
- (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(vi) ORIGINAL SOURCE:
- (C) INDIVIDUAL/ISOLATE: Human

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

```
GTGCCTGCAG AGACCCGTCT GGTGCACTCT GATTCTCCAC TTGCCTGTTG CATGTCCTCG     60
TTCCCTTGTT TCTCACCACC TCTTGGGTTG CCATGTGCGT TTCCTGCCGA GTGTGTGTTG    120
ATCCTCTCGT TGCCTCCTGG TCACTGGGCA TTTGCTTTTA TTTCTCTTTG CTTAGTGTTA    180
CCCCCTGATC TTTTTATTGT CGTTGTTTGC TTTTGTTTAT TGAGACAGTC TCACTCTGTC    240
ACCCAGGCTG GAGTGTAATG GCACAATCTC GGCTCACTGC AACCTCTGCC TCCTCGGTTC    300
AAGCAGTTCT CATTCCTCAA CCTCATGAGT AGCTGGGATT ACAGGCGCCC ACCACCACGC    360
CTGGCTAATT TTTGTATTTT TAGTAGAGAT AGGCTTTCAC CATGTTGGCC AGGCTGGTCT    420
CAAACTCCTG ACCTCAAGTG ATCTGCCCGC CTTGGCCTCC CACAGTGCTG GGATTACAGG    480
TGCAAGCCAC CGTGCCCGGC ATACCTTGAT CTTTTAAAAT GAAGTCTGAA ACATTGCTAC    540
CCTTGTCCTG AGCAATAAGA CCCTTAGTGT ATTTTAGCTC TGGCCACCCC CCAGCCTGTG    600
TGCTGTTTTC CCTGCTGACT TAGTTCTATC TCAGGCATCT TGACACCCCC ACAAGCTAAG    660
CATTATTAAT ATTGTTTTCC GTGTTGAGTG TTTCTTTAGC TTTGCCCCCG CCCTGCTTTT    720
CCTCCTTTGT TCCCCGTCTG TCTTCTGTCT CAGGCCCGCC GTCTGGGGTC CCCTTCCTTG    780
TCCTTTGCGT GGTTCTTCTG TCTTGTTATT GCTGGTAAAC CCCAGCTTTA CCTGTGCTGG    840
CCTCCATGGC ATCTAGCGAC GTCCGGGGAC CTCTGCTTAT GATGCACAGA TGAAGATGTG    900
GAGACTCACG AGGAGGGCGG TCATCTTGGC CCGTGAGTGT CTGGAGCACC ACGTGGCCAG    960
CGTTCCTTAG CCAGGGTTGG CTGTGTTCCG GCCGCAGAGC ACCGTCTGCG TGAGGAGATC   1020
CTGGCCAAGT TCCTGCACTG GCTGATGAGT GTGTACGTCG TCGAGCTGCT CAGGTCTTTC   1080
TTTTATGTCA CGGAGACCAC GTTTCAAAAG AACAGGCTCT TTTTCTACCG GAAGAGTGTC   1140
TGGAGCAAGT TGC                                                      1153
```

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 412 base pairs
(B) TYPE: Nucleotide
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(vi) ORIGINAL SOURCE:
(C) INDIVIDUAL/ISOLATE: Human

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

CAGAGCCCTG GTCCTCCTGT CTCCATCGTC ACGTGGGCAC ACGTGGCTTT TCGCTCAGGA 60

CGTCGAGTGG ACACGGTGAT CTCTGCCTCT GCTCTCCCTC CTGTCCAGTT TGCATAAACT 120

TACGAGGTTC ACCTTCACGT TTTGATGGAC ACGCGGTTTC CAGGCACCGA GGCCAGAGCA 180

GTGAACAGAG GAGGCTGGGC GCGGCAGTGG AGCCGGGTTG CCGGCAATGG GGAGAAGTGT 240

CTGGAAGCAC AGACGCTCTG GCGAGGGTGC CTGCAGAGAC CCGCCTGGTG CACTCTGATT 300

CTCCACTTGC CTGTTGCATG TCCTCGTTCC CTTGTTTCTC ACCACCTCTT GGGTTGCCAT 360

GTGCGTTTCC TGCCGAGTGT GTGTTGATCC TCTCGTTGCC TCCTGGTCAC TG 412

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1012 base pairs
(B) TYPE: Nucleotide
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(ii) MOLCULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(vi) ORIGINAL SOURCE:
(C) INDIVIDUAL/ISOLATE: Human

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

GGGGTCCTGG GCCCACCCGG GCAGGACGCG TGGACCGAGT GACCGTGGTT TCTGTGTGGT 60

GTCACCTGCC AGACCCGCCG AAGAAGCCAC CTCTTTGGAG GGTGCGCTCT CTGGCACGCG 120

CCACTCCCAC CCATCCGTGG GCCGCCAGCA CCACGCGGGC CCCCCATCCA CATCGCGGCC 180

ACCACGTCCC TGGGACACGC CTTGTCCCCC GGTGTACGCC GAGACCAAGC ACTTCCTCTA 240

CTCCTCAGGC GACAAGGAGC AGCTGCGGCC CTCCTTCCTA CTCAGCTCTC TGAGGCCCAG 300

CCTGACTGGC GCTCGGAGGC TCGTGGAGAC CATCTTTCTG GGTTCCAGGC CCTGGATGCC 360

AGGGACTCCC CGCAGGTTGC CCCGCCTGCC CCAGCGCTAC TGGCAAATGC GGCCCCTGTT 420

TCTGGAGCTG CTTGGGAACC ACGCGCAGTG CCCCTACGGG GTGCTCCTCA AGACGCACTG 480

CCCGCTGCGA GCTGCGGTCA CCCCAGCAGC CGGTGTCTGT GCCCGGGAGA AGCCCCAGGG 540

CTCTGTGGCG GCCCCCGAGG AGGAGGACAC AGACCCCCGT CGCCTGGTGC AGCTGCTCCG 600

CCAGCACAGC AGCCCCTGGC AGGTGTACGG CTTCGTGCGG GCCTGCCTGC GCCGGCTGGT 660

GCCCCCAGGC CTCTGGGGCT CCAGGCACAA CGAACGCCGC TTCCTCAGGA ACACCAAGAA 720

GTTCATCTCC CTGGGGAAGC ATGCCAAGCT CTCGCTGCAG GAGCTGACGT GGAAGATGAG 780

CGTGCGGGAC TGCGCTTGGC TGCGCAGGAG CCCAGGTGAG GAGGTGGTGG CCGTCGAGGG 840

CCCAGGCCCC AGAGCTGAAT GCAGTAGGGG CTCAGAAAAG GGGGCAGGCA GAGCCCTGGT 900

CCTCCTGTCT CCATCGTCAC GTGGGCACAC GTGGCTTTTC GCTCAGGACG TCGAGTGGAC 960

ACGGTGATCT CTGCCTCTGC TCTCCCTCCT GTCCAGTTTG CATAAACTTA CG 1012

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3972 base pairs
(B) TYPE: Nucleotide
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(vi) ORIGINAL SOURCE:
(C) INDIVIDUAL/ISOLATE: Human

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

GAATTCGCGG CCGCGTCGAC GTTTCAGGCA GCGCTGCGTC CTGCTGCGCA CGTGGGAAGC 60

CCTGGCCCCG GCCACCCCCG CGATGCCGCG CGCTCCCCGC TGCCGAGCCG TGCGCTCCCT 120

GCTGCGCAGC CACTACCGCG AGGTGCTGCC GCTGGCCACG TTCGTGCGGC GCCTGGGGCC 180

CCAGGGCTGG CGGCTGGTGC AGCGCGGGGA CCCGGCGGCT TTCCGCGCGC TGGTGGCCCA 240

GTGCCTGGTG TGCGTGCCCT GGGACGCACG GCCGCCCCCC GCCGCCCCCT CCTTCCGCCA 300

GGTGTCCTGC CTGAAGGAGC TGGTGGCCCG AGTGCTGCAG AGGCTGTGCG AGCGCGGCGC 360

GAAGAACGTG CTGGCCTTCG GCTTCGCGCT GCTGGACGGG GCCCGCGGGG GCCCCCCCGA 420

GGCCTTCACC ACCAGCGTGC GCAGCTACCT GCCCAACACG GTGACCGACG CACTGCGGGG 480

GAGCGGGGCG TGGGGGCTGC TGCTGCGCCG CGTGGGCGAC GACGTGCTGG TTCACCTGCT 540

GGCACGCTGC GCGCTCTTTG TGCTGGTGGC TCCCAGCTGC GCCTACCAGG TGTGCGGGCC 600

GCCGCTGTAC CAGCTCGGCG CTGCCACTCA GGCCCGGCCC CCGCCACACG CTAGTGGACC 660

CCGAAGGCGT CTGGGATGCG AACGGGCCTG GAACCATAGC GTCAGGGAGG CCGGGGTCCC 720

CCTGGGCCTG CCAGCCCCGG GTGCGAGGAG GCGCGGGGGC AGTGCCAGCC GAAGTCTGCC 780

GTTGCCCAAG AGGCCCAGGC GTGGCGCTGC CCCTGAGCCG GAGCGGACGC CCGTTGGGCA 840

GGGGTCCTGG GCCCACCCGG GCAGGACGCG TGGACCGAGT GACCGTGGTT TCTGTGTGGT 900

GTCACCTGCC AGACCCGCCG AAGAAGCCAC CTCTTTGGAG GGTGCGCTCT CTGGCACGCG 960

CCACTCCCAC CCATCCGTGG GCCGCCAGCA CCACGCGGGC CCCCCATCCA CATCGCGGCC 1020

ACCACGTCCC TGGGACACGC CTTGTCCCCC GGTGTACGCC GAGACCAAGC ACTTCCTCTA 1080
CTCCTCAGGC GACAAGGAGC AGCTGCGGCC CTCCTTCCTA CTCAGCTCTC TGAGGCCCAG 1140
CCTGACTGGC GCTCGGAGGC TCGTGGAGAC CATCTTTCTG GGTTCCAGGC CCTGGATGCC 1200
AGGGACTCCC CGCAGGTTGC CCCGCCTGCC CCAGCGCTAC TGGCAAATGC GGCCCCTGTT 1260
TCTGGAGCTG CTTGGGAACC ACGCGCAGTG CCCCTACGGG GTGCTCCTCA AGACGCACTG 1320
CCCGCTGCGA GCTGCGGTCA CCCCAGCAGC CGGTGTCTGT GCCCGGGAGA AGCCCCAGGG 1380
CTCTGTGGCG GCCCCCGAGG AGGAGGACAC AGACCCCCGT CGCCTGGTGC AGCTGCTCCG 1440
CCAGCACAGC AGCCCCTGGC AGGTGTACGG CTTCGTGCGG GCCTGCCTGC GCCGGCTGGT 1500
GCCCCCAGGC CTCTGGGGCT CCAGGCACAA CGAACGCCGC TTCCTCAGGA ACACCAAGAA 1560
GTTCATCTCC CTGGGGAAGC ATGCCAAGCT CTCGCTGCAG GAGCTGACGT GGAAGATGAG 1620
CGTGCGGGAC TGCGCTTGGC TGCGCAGGAG CCCAGGTGAG GAGGTGGTGG CCGTCGAGGG 1680
CCCAGGCCCC AGAGCTGAAT GCAGTAGGGG CTCAGAAAAG GGGGCAGGCA GAGCCCTGGT 1740
CCTCCTGTCT CCATCGTCAC GTGGGCACAC GTGGCTTTTC GCTCAGGACG TCGAGTGGAC 1800
ACGGTGATCT CTGCCTCTGC TCTCCCTCCT GTCCAGTTTG CATAAACTTA CGAGGTTCAC 1860
CTTCACGTTT TGATGGACAC GCGGTTTCCA GGCGCCGAGG CCAGAGCAGT GAACAGAGGA 1920
GGCTGGGCGC GGCAGTGGAG CCGGGTTGCC GGCAATGGGG AGAAGTGTCT GGAAGCACAG 1980
ACGCTCTGGC GAGGGTGCCT GCAGGGGTTG GCTGTGTTCC GGCCGCAGAG CACCGTCTGC 2040
GTGAGGAGAT CCTGGCCAAG TTCCTGCACT GGCTGATGAG TGTGTACGTC GTCGAGCTGC 2100
TCAGGTCTTT CTTTTATGTC ACGGAGACCA CGTTTCAAAA GAACAGGCTC TTTTTCTACC 2160
GGAAGAGTGT CTGGAGCAAG TTGCAAAGCA TTGGAATCAG ACAGCACTTG AAGAGGGTGC 2220
AGCTGCGGGA GCTGTCGGAA GCAGAGGTCA GGCAGCATCG GGAAGCCAGG CCCGCCCTGC 2280
TGACGTCCAG ACTCCGCTTC ATCCCCAAGC CTGACGGGCT GCGGCCGATT GTGAACATGG 2340
ACTACGTCGT GGGAGCCAGA ACGTTCCGCA GAGAAAAGAG GGTGGCTGTG CTTTGGTTTA 2400
ACTTCCTTTT TAAACAGAAG TGCGTTTGAG CCCCACATTT GGTATCAGCT TAGATGAAGG 2460
GCCCGGAGGA GGGGCCACGG GACACAGCCA GGGCCATGGC ACGGCGCCAA CCCATTTGTG 2520
CGCACGGTGA GGTGGCCGAG GTGCCGGTGC CTCCAGAAAA GCAGCGTGGG GGTGTAGGGG 2580
GAGCTCCTGG GGCAGGGACA GGCTCTGAGG ACCACAAGAA GCAGCTGGGC CAGGGCCTGG 2640
ATGCAGCACG GCCCGAGCGG GTGGGGGCCC ACCACGCCAT TCTGGTCAAA GGTGTTGTAG 2700
TCGTAATAGC CGGCCCAGGC GCTCTGAACC TTCAGAGTCT CAAAAGCTGG GACCCTCAGG 2760
GCCAAATGGG GCCACACCTT GTCCTGGAAG AAATCATGGT CCACTTCCAG GTTCGCCGGG 2820
TCCGGTTCTT CCTGCTCAGT GGGGCTACGA CCACCTAGGT AGTTGCTACC TAATCCTTCC 2880

CGGCGAAAAT AGGCTCCACT GGTGTCTGCA ACAAGCGGAG TCTCTAGGCC TGGTCCCTGG 2940

GGGCAGTGCC ACACATACAC ATACCTTTTC CTCGGCTCCA CAGGTAGCTT GGTGCCCTGC 3000

AGGGTGCCAG GCGGCCCCTC TCCAACACCA GCCAGTGCTG CGATTTGCGC AGACCAGGCT 3060

CCGGCTGCGT TGATCACAAT GGCGCATTCC ACAGGCTGGT ACTCCAGGCT GCGGTCCATC 3120

TTCACATGGA CTTCATGGAT CCTTTTCAAG ACCACCGCTT TGTCATCTGT GGTCAACATG 3180

CGTTGAGATG AAGAGACAAA ACGTGTCACC TCTCCCTGGC AGAAAAGGAC TCCCAAGGAC 3240

TGGACCTTTC GCCGAAGCCC CTGGAGCAGA CACCAGGGGT CAAACCAACC TTCGTCCTCC 3300

ATCCCATAAG ACGCCAAAGC CACTCCCTCT GTGTTTATCC AGGGAAACTT GTTCCGAAGC 3360

TGATCAGGAG ACATCAGAGA AACTTTGGCT CCCTCCTGCC TCTGCACTTT CACGTTGCTC 3420

TCCATGGCTG CAGCATCCTT TTCTGAAGCC AGCAAGAGGT AGCCCGAGGG GTTGAACCGG 3480

AGGTCCAGGG GAGGAGCATC GACTACGGCC AGGTACTCAT TGATGTTCCG TAGAAAGCTG 3540

GCTGAAAAGA GGGAGAGCTG GATGTTCTCA GGCAATGAGA ACTGCTGACA AATCCCACCT 3600

ACTGAGAGCC CAGTGGAGGC CTGTGAATAC GTGTGGTCCC GTTCCACCAC TAGCACTCGA 3660

ATAGCACCTC GTCTGCTCTC CAGCTTCTTC AGCCAATAGG CCACAGACAA GCCAAGCACC 3720

CCACCTCCCA CGATCACCAC ATCCGAGTGC TCGGGAGGCA GGTGGCTGGT GTCTTGCAGT 3780

AGATCACAGG ACCTTCCAGG CAGGATCGAC TTGATCTTCT TCTTAATCTC AGACACCTTT 3840

CCATCCCAGT CCAGAGAAAA GCCTCCTCTG CGCGTGCCTG GCCTCCGGGT CAAGAGGCCC 3900

CGGCCCATGC CGTGCGGCAG AACCCTCCGA ATCATAGCCC CTCTGAGCCC GGGTCGACGC 3960

GGCCGCGAAT TC 3972

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2089 base pairs
(B) TYPE: Nucleotide
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: cDNA

(v) FRAGMENT TYPE: Linear

(vi) ORIGINAL SOURCE:
(C) INDIVIDUAL/ISOLATE: Human

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

CCGGAAGAGT GTCTGGAGCA AGTTGCAAAG CATTGGAATC AGACAGCACT TGAAGAGGGT 60

GCAGCTGCGG GAGCTGTCGG AAGCAGAGGT CAGGCAGCAT CGGGAAGCCA GGCCCGCCCT 120

GCTGACGTCC AGACTCCGCT TCATCCCCAA GCCTGACGGG CTGCGGCCGA TTGTGAACAT 180

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GGACTACGTC GTGGGAGCCA GAACGTTCCG CAGAGAAAAG AGGGCCGAGC GTCTCACCTC    240
GAGGGTGAAG GCACTGTTCA GCGTGCTCAA CTACGAGCGG GCGCGGCGCC CCGGCCTCCT    300
GGGCGCCTCT GTGCTGGGCC TGGACGATAT CCACAGGGCC TGGCGCACCT TCGTGCTGCG    360
TGTGCGGGCC CAGGACCCGC CGCCTGAGCT GTACTTTGTC AAGGTGGATG TGACGGGCGC    420
GTACGACACC ATCCCCCAGG ACAGGCTCAC GGAGGTCATC GCCAGCATCA TCAAACCCCA    480
GAACACGTAC TGCGTGCGTC GGTATGCCGT GGTCCAGAAG GCCGCCCATG GGCACGTCCG    540
CAAGGCCTTC AAGAGCCACG TCTCTACCTT GACAGACCTC CAGCCGTACA TGCGACAGTT    600
CGTGGCTCAC CTGCAGGAGA CCAGCCCGCT GAGGGGTGCC GTCGTCATCG AGCAGAGCTC    660
CTCCCTGAAT GAGGCCAGCA GTGGCCTCTT CGACGTCTTC CTACGCTTCA TGTGCCACCA    720
CGCCGTGCGC ATCAGGGGCA AGTCCTACGT CCAGTGCCAG GGGATCCCGC AGGGCTCCAT    780
CCTCTCCACG CTGCTCTGCA GCCTGTGCTA CGGCGACATG GAGAACAAGC TGTTTGCGGG    840
GATTCGGCGG GACGGGCTGC TCCTGCGTTT GGTGGATGAT TTCTTGTTGG TGACACCTCA    900
CCTCACCCAC GCGAAAACCT TCCTCAGGAC CCTGGTCCGA GGTGTCCCTG AGTATGGCTG    960
CGTGGTGAAC TTGCGGAAGA CAGTGGTGAA CTTCCCTGTA GAAGACGAGG CCCTGGGTGG   1020
CACGGCTTTT GTTCAGATGC CGGCCCACGG CCTATTCCCC TGGTGCGGCC TGCTGCTGGA   1080
TACCCGGACC CTGGAGGTGC AGAGCGACTA CTCCAGCTAT GCCCGGACCT CCATCAGAGC   1140
CAGTCTCACC TTCAACCGCG GCTTCAAGGC TGGGAGGAAC ATGCGTCGCA AACTCTTTGG   1200
GGTCTTGCGG CTGAAGTGTC ACAGCCTGTT TCTGGATTTG CAGGTGAACA GCCTCCAGAC   1260
GGTGTGCACC AACATCTACA AGATCCTCCT GCTGCAGGCG TACAGGTTTC ACGCATGCGT   1320
GCTGCAGCTC CCATTTCATC AGCAAGTTTG GAAGAACCCC ACATTTTTCC TGCGCGTCAT   1380
CTCTGACACG GCCTCCCTCT GCTACTCCAT CCTGAAAGCC AAGAACGCAG GTATGTGCAG   1440
GTGCCTGGCC TCAGTGGCAG CAGTGCCTGC CTGCTGGTGT TAGTGTGTCA GGAGACTGAG   1500
TGAATCTGGG CTTAGGAAGT TCTTACCCCT TTTCGCATCA GGAAGTGGTT TAACCCAACC   1560
ACTGTCAGGC TCGTCTGCCC GCCCTCTCGT GGGGTGAGCA GAGCACCTGA TGGAAGGGAC   1620
AGGAGCTGTC TGGGAGCTGC CATCCTTCCC ACCTTGCTCT GCCTGGGGAA GCGCTGGGGG   1680
GCCTGGTCTC TCCTGTTTGC CCCATGGTGG GATTTGGGGG GCCTGGCCTC TCCTGTTTGC   1740
CCTGTGGTGG GATTGGGCTG TCTCCCGTCC ATGGCACTTA GGGCCCTTGT GCAAACCCAG   1800
GCCAAGGGCT TAGGAGGAGG CCAGGCCCAG GCTACCCCAC CCCTCTCAGG AGCAGAGGCC   1860
GCGTATCACC ACGACAGAGC CCCGCGCCGT CCTCTGCTTC CCAGTCACCG TCCTCTGCCC   1920
CTGGACACTT TGTCCAGCAT CAGGGAGGTT TCTGATCCGT CTGAAATTCA AGCCATGTCG   1980
AACCTGCGGT CCTGAGCTTA ACAGCTTCTA CTTTCTGTTC TTTCTGTGTT GTGGAGACCC   2040
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TGAGAAGGAC CCTGGGAGCT CTGGGAATTT GGAGTGACCA AAGGTGTGC 2089

Patent Claims

1. Catalytically active human telomerase subunit, its functional equivalents, its variants and its catalytically active fragments.

2. Telomerase according to Claim 1, comprising the amino acid sequence depicted in Fig. 1b or its functional equivalents.

3. Nucleic acid sequences encoding compounds according to Claims 1 and 2 and their functional equivalents.

4. Nucleic acid sequences according to Claim 3, comprising the DNA sequence depicted in Fig. 1a or its functional equivalents.

5. Antisense nucleic acids binding to the nucleic acid sequence according to Claim 3 or 4.

6. Antibodies against telomerase according to Claims 1 and 2, where appropriate labelled with one or more labels.

7. Use of nucleic acid sequences according to Claims 3 and 4 for preparing telomerase.

8. Use of antibodies according to Claim 6 for diagnosis.

9. Use of antibodies according to Claim 6 for preparing medicaments.

10. Vector comprising a nucleic acid sequence, in particular DNA, according to Claims 3 and 4.

11. Microorganisms harbouring the vector according to Claim 10.

12. Screening assay for identifying modulators of human telomerase comprising the telomerase according to Claims 1 and 2.

13. Process for preparing the telomerase according to Claims 1 and 2, characterized in that the microorganism according to Claim 11 is cultured and the telomerase is isolated.

Catalytic subunit of human telomerase and its diagnostic and therapeutic use

Abstract

This invention relates to the nucleotide sequence, and the protein sequence which is deduced from it, which encodes the catalytic subunit of human telomerase. In addition, this invention relates to methods which involve a pharmaceutical, diagnostic or therapeutic use of this gene/protein, especially in the treatment of cancer and ageing.

Fig.1

```
GTTTCAGGCA GCGCTGCGTC CTGCTGCGCA CGTGGGAAGC CCTGGCCCCG GCCACCCCCG CGATGCCGCG   70
CGCTCCCCGC TGCCGAGCCG TGCGCTCCCT GCTGCGCAGC CACTACCGCG AGGTGCTGCC GCTGGCCACG  140
TTCGTGCGGC GCCTGGGGCC CCAGGGCTGG CGGCTGGTGC AGCGCGGGGA CCCGGCGGCT TTCCGCGCGC  210
TGGTGGCCCA GTGCCTGGTG TGCGTGCCCT GGGACGCACG GCCGCCCCCC GCCGCCCCCT CCTTCCGCCA  280
GGTGTCCTGC CTGAAGGAGC TGGTGGCCCG AGTGCTGCAG AGGCTGTGCG AGCGCGGCGC GAAGAACGTG  350
CTGGCCTTCG GCTTCGCGCT GCTGGACGGG GCCCGCGGGG GCCCCCCCGA GGCCTTCACC ACCAGCGTGC  420
GCAGCTACCT GCCCAACACG GTGACCGACG CACTGCGGGG GAGCGGGGCG TGGGGGCTGC TGCTGCGCCG  490
CGTGGGCGAC GACGTGCTGG TTCACCTGCT GGCACGCTGC GCGCTCTTTG TGCTGGTGGC TCCCAGCTGC  560
GCCTACCAGG TGTGCGGGCC GCCGCTGTAC CAGCTCGGCG CTGCCACTCA GGCCCGGCCC CCGCCACACG  630
CTAGTGGACC CCGAAGGCGT CTGGGATGCG AACGGGCCTG GAACCATAGC GTCAGGGAGG CCGGGGTCCC  700
CCTGGGCCTG CCAGCCCCGG GTGCGAGGAG GCGCGGGGGC AGTGCCAGCC GAAGTCTGCC GTTGCCCAAG  770
AGGCCCAGGC GTGGCGCTGC CCCTGAGCCG GAGCGGACGC CCGTTGGGCA GGGGTCCTGG GCCCACCCGG  840
GCAGGACGCG TGGACCGAGT GACCGTGGTT TCTGTGTGGT GTCACCTGCC AGACCCGCCG AAGAAGCCAC  910
CTCTTTGGAG GGTGCGCTCT CTGGCACGCG CCACTCCCAC CCATCCGTGG GCCGCCAGCA CCACGCGGGC  980
CCCCCATCCA CATCGCGGCC ACCACGTCCC TGGGACACGC CTTGTCCCCC GGTGTACGCC GAGACCAAGC 1050
ACTTCCTCTA CTCCTCAGGC GACAAGGAGC AGCTGCGGCC CTCCTTCCTA CTCAGCTCTC TGAGGCCCAG 1120
CCTGACTGGC GCTCGGAGGC TCGTGGAGAC CATCTTTCTG GGTTCCAGGC CCTGGATGCC AGGGACTCCC 1190
CGCAGGTTGC CCCGCCTGCC CCAGCGCTAC TGGCAAATGC GGCCCCTGTT TCTGGAGCTG CTTGGGAACC 1260
ACGCGCAGTG CCCCTACGGG GTGCTCCTCA AGACGCACTG CCCGCTGCGA GCTGCGGTCA CCCCAGCAGC 1330
CGGTGTCTGT GCCCGGGAGA AGCCCCAGGG CTCTGTGGCG GCCCCCGAGG AGGAGGACAC AGACCCCCGT 1400
CGCCTGGTGC AGCTGCTCCG CCAGCACAGC AGCCCCTGGC AGGTGTACGG CTTCGTGCGG GCCTGCCTGC 1470
GCCGGCTGGT GCCCCCAGGC CTCTGGGGCT CCAGGCACAA CGAACGCCGC TTCCTCAGGA ACACCAAGAA 1540
GTTCATCTCC CTGGGGAAGC ATGCCAAGCT CTCGCTGCAG GAGCTGACGT GGAAGATGAG CGTGCGGGAC 1610
TGCGCTTGGC TGCGCAGGAG CCCAGGGGTT GGCTGTGTTC CGGCCGCAGA GCACCGTCTG CGTGAGGAGA 1680
TCCTGGCCAA GTTCCTGCAC TGGCTGATGA GTGTGTACGT CGTCGAGCTG CTCAGGTCTT TCTTTTATGT 1750
CACGGAGACC ACGTTTCAAA AGAACAGGCT CTTTTTCTAC CGGAAGAGTG TCTGGAGCAA GTTGCAAAGC 1820
ATTGGAATCA GACAGCACTT GAAGAGGGTG CAGCTGCGGG AGCTGTCGGA AGCAGAGGTC AGGCAGCATC 1890
GGGAAGCCAG GCCCGCCCTG CTGACGTCCA GACTCCGCTT CATCCCCAAG CCTGACGGGC TGCGGCCGAT 1960
TGTGAACATG GACTACGTCG TGGGAGCCAG AACGTTCCGC AGAGAAAAGA GGGCCGAGCG TCTCACCTCG 2030
AGGGTGAAGG CACTGTTCAG CGTGCTCAAC TACGAGCGGG CGCGGCGCCC CGGCCTCCTG GGCGCCTCTG 2100
TGCTGGGCCT GGACGATATC CACAGGGCCT GGCGCACCTT CGTGCTGCGT GTGCGGGCCC AGGACCCGCC 2170
GCCTGAGCTG TACTTTGTCA AGGTGGATGT GACGGGCGCG TACGACACCA TCCCCCAGGA CAGGCTCACG 2240
GAGGTCATCG CCAGCATCAT CAAACCCCAG AACACGTACT GCGTGCGTCG GTATGCCGTG GTCCAGAAGG 2310
CCGCCCATGG GCACGTCCGC AAGGCCTTCA AGAGCCACGT CTCTACCTTG ACAGACCTCC AGCCGTACAT 2380
GCGACAGTTC GTGGCTCACC TGCAGGAGAC CAGCCCGCTG AGGGATGCCG TCGTCATCGA GCAGAGCTCC 2450
TCCCTGAATG AGGCCAGCAG TGGCCTCTTC GACGTCTTCC TACGCTTCAT GTGCCACCAC GCCGTGCGCA 2520
TCAGGGGCAA GTCCTACGTC CAGTGCCAGG GGATCCCGCA GGGCTCCATC CTCTCCACGC TGCTCTGCAG 2590
CCTGTGCTAC GGCGACATGG AGAACAAGCT GTTTGCGGGG ATTCGGCGGG ACGGGCTGCT CCTGCGTTTG 2660
GTGGATGATT TCTTGTTGGT GACACCTCAC CTCACCCACG CGAAAACCTT CCTCAGGACC CTGGTCCGAG 2730
GTGTCCCTGA GTATGGCTGC GTGGTGAACT TGCGGAAGAC AGTGGTGAAC TTCCCTGTAG AAGACGAGGC 2800
CCTGGGTGGC ACGGCTTTTG TTCAGATGCC GGCCCACGGC CTATTCCCCT GGTGCGGCCT GCTGCTGGAT 2870
ACCCGGACCC TGGAGGTGCA GAGCGACTAC TCCAGCTATG CCCGGACCTC CATCAGAGCC AGTCTCACCT 2940
TCAACCGCGG CTTCAAGGCT GGGAGGAACA TGCGTCGCAA ACTCTTTGGG GTCTTGCGGC TGAAGTGTCA 3010
CAGCCTGTTT CTGGATTTGC AGGTGAACAG CCTCCAGACG GTGTGCACCA ACATCTACAA GATCCTCCTG 3080
CTGCAGGCGT ACAGGTTTCA CGCATGTGTG CTGCAGCTCC CATTTCATCA GCAAGTTTGG AAGAACCCCA 3150
CATTTTTCCT GCGCGTCATC TCTGACACGG CCTCCCTCTG CTACTCCATC CTGAAAGCCA AGAACGCAGG 3220
GATGTCGCTG GGGGCCAAGG GCGCCGCCGG CCCTCTGCCC TCCGAGGCCG TGCAGTGGCT GTGCCACCAA 3290
GCATTCCTGC TCAAGCTGAC TCGACACCGT GTCACCTACG TGCCACTCCT GGGGTCACTC AGGACAGCCC 3360
AGACGCAGCT GAGTCGGAAG CTCCCGGGGA CGACGCTGAC TGCCCTGGAG GCCGCAGCCA ACCCGGCACT 3430
GCCCTCAGAC TTCAAGACCA TCCTGGACTG ATGGCCACCC GCCCACAGCC AGGCCGAGAG CAGACACCAG 3500
CAGCCCTGTC ACGCCGGGCT CTACGTCCCA GGGAGGGAGG GGCGGCCCAC ACCCAGGCCC GCACCGCTGG 3570
GAGTCTGAGG CCTGAGTGAG TGTTTGGCCG AGGCCTGCAT GTCCGGCTGA AGGCTGAGTG TCCGGCTGAG 3640
GCCTGAGCGA GTGTCCAGCC AAGGGCTGAG TGTCCAGCAC ACCTGCCGTC TTCACTTCCC CACAGGCTGG 3710
CGCTCGGCTC CACCCCAGGG CCAGCTTTTC CTCACCAGGA GCCCGGCTTC CACTCCCCAC ATAGGAATAG 3780
TCCATCCCCA GATTCGCCAT TGTTCACCCC TCGCCCTGCC CTCCTTTGCC TTCCACCCCC ACCATCCAGG 3850
TGGAGACCCT GAGAAGGACC CTGGGAGCTC TGGGAATTTG GAGTGACCAA AGGTGTGCCC TGTACACAGG 3920
CGAGGACCCT GCACCTGGAT GGGGGTCCCT GTGGGTCAAA TTGGGGGGAG GTGCTGTGGG AGTAAAATAC 3990
TGAATATATG AGTTTTTCAG TTTTGAAAAA AAAAAAAAAA AAAAAAAAAA AA                   4042
```

Fig. 2

MPRAPRCRAV	RSLLRSHYRE	VLPLATFVRR	LGPQGWRLVQ	RGDPAAFRAL	50
VAQCLVCVPW	DARPPPAAPS	FRQVSCLKEL	VARVLQRLCE	RGAKNVLAFG	100
FALLDGARGG	PPEAFTTSVR	SYLPNTVTDA	LRGSGAWGLL	LRRVGDDVLV	150
HLLARCALFV	LVAPSCAYQV	CGPPLYQLGA	ATQARPPPHA	SGPRRRLGCE	200
RAWNHSVREA	GVPLGLPAPG	ARRRGGSASR	SLPLPKRPRR	GAAPEPERTP	250
VGQGSWAHPG	RTRGPSDRGF	CVVSPARPAE	EATSLEGALS	GTRHSHPSVG	300
RQHHAGPPST	SRPPRPWDTP	CPPVYAETKH	FLYSSGDKEQ	LRPSFLLSSL	350
RPSLTGARRL	VETIFLGSRP	WMPGTPRRLP	RLPQRYWQMR	PLFLELLGNH	400
AQCPYGVLLK	THCPLRAAVT	PAAGVCAREK	PQGSVAAPEE	EDTDPRRLVQ	450
LLRQHSSPWQ	VYGFVRACLR	RLVPPGLWGS	RHNERRFLRN	TKKFISLGKH	500
AKLSLQELTW	KMSVRDCAWL	RRSPGVGCVP	AAEHRLREEI	LAKFLHWLMS	550
VYVVELLRSF	FYVTETTFQK	NRLFFYRKSV	WSKLQSIGIR	QHLKRVQLRE	600
LSEAEVRQHR	EARPALLTSR	LRFIPKPDGL	RPIVNMDYVV	GARTFRREKR	650
AERLTSRVKA	LFSVLNYERA	RRPGLLGASV	LGLDDIHRAW	RTFVLRVRAQ	700
DPPPELYFVK	VDVTGAYDTI	PQDRLTEVIA	SIIKPQNTYC	VRRYAVVQKA	750
AHGHVRKAFK	SHVSTLTDLQ	PYMRQFVAHL	QETSPLRDAV	VIEQSSSLNE	800
ASSGLFDVFL	RFMCHHAVRI	RGKSYVQCQG	IPQGSILSTL	LCSLCYGDME	850
NKLFAGIRRD	GLLLRLVDDF	LLVTPHLTHA	KTFLRTLVRG	VPEYGCVVNL	900
RKTVVNFPVE	DEALGGTAFV	QMPAHGLFPW	CGLLLDTRTL	EVQSDYSSYA	950
RTSIRASLTF	NRGFKAGRNM	RRKLFGVLRL	KCHSLFLDLQ	VNSLQTVCTN	1000
IYKILLLQAY	RFHACVLQLP	FHQQVWKNPT	FFLRVISDTA	SLCYSILKAK	1050
NAGMSLGAKG	AAGPLPSEAV	QWLCHQAFLL	KLTRHRVTYV	PLLGSLRTAQ	1100
TQLSRKLPGT	TLTALEAAAN	PALPSDFKTI	LD		1132

Fig. 3

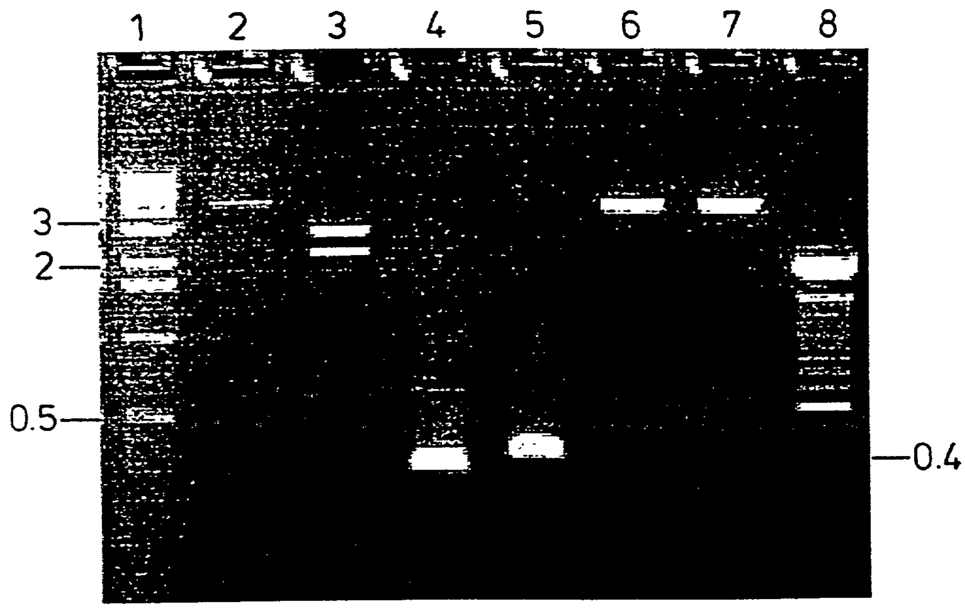

Fig. 4

Lipman-Pearson Protein Alignment

Ktuple: 2; Gap Penalty: 4; Gap Length Penalty: 12

Seq1(1>129) PHTC.PRO	Seq2(1>150) P123.PRO	Similarity Index	Gap Number	Gap Length	Consensus Length
(2>124)	(1>117)	31.5	4	6	123

```
                      10        20        30        40        50        60        70        80
PHTC.PRO KFLHWLMSVYVVELLRSFFYVTETTFQKNRLFFYRKSVWSKLQSIGIRQHLKRVQLRDVSEAEVRQHREARPALLTSRLR
         K:L:W:..  VV.L:R.FFYVTE     :: ::YRK::W. :  .::I .:LK:  L :V E EV :::. . :: .::LR
P123.PRO KLLRWIFEDLVVSLIRCFFYVTEQQKSYSKTYYYRKNIWDVIMKMSI-ADLKKETLAEVQEKEV-EEWKKSLGFAPGKLR
                  10        20        30        40         50        60         70

                      90       100       110       120
PHTC.PRO FIPKPDGLRPIVNMDYVVGARTFRREKRAERLTSRVKALFSVLNYERA
         :IPK ..:RPI  M.:   . :. ..:::::LT:..K L S L   :
P123.PRO LIPKKTTFRPI--MTF--NKKIVNSDRKTTKLTTNTKLLNSHLMLKTL
          80          90          100      110       120
```

Fig. 5

Lipman-Pearson Protein Alignment

Ktuple: 2; Gap Penalty: 4; Gap Length Penalty: 12

Seq1(1>150) P123.PRO	Seq2(1>150) EST2P.PRO	Similarity Index	Gap Number	Gap Length	Consensus Length
(2>148)	(1>146)	21.6	4	5	149

```
                  10        20        30        40        50        60        70        80
P123.PRO  LLRWIFEDLVVSLIRCFFYVTEQQKSYSKTYYYRKNIWDVIMKMSIADLKKETLAEVQEKEVEEWKKSLGFAPGKLRLIP
          ::.W:F :L: .:I: FFY TE  .: : .Y: R.:.W: ::.  I.:  K. L.E :    ::  .  .F..:K:R:IP
EST2P.PRO FISWLFRQLIPKIIQTFFYCTEISSTVTIVYF-RHDTWNKLITPFIVEYFKTYLVENNVCRNHNSYTLSNFNHSKMRIIP
                    10        20        30        40        50        60        70
                    90        100       110       120       130       140       150
P123.PRO  KKT--TFRPIMTFNKKIVNSDRKTTKLTTNTKLLNSHLMLKTLKNRMFKDPFGFAVFNYDDVMKKYEEFVC
          KK:  .FR I .  :    :.: ..K . :. :  :: :L. L:N:.  .:F.  ::: .:: .: .EF
EST2P.PRO KKSNNEFRIIAIPCRGADEEEFTIYKENHKNAIQPTQKILEYLRNKR-PTSFT-KIYSPTQIADRIKEFKQ
          80        90        100       110       120       130       140
```

Fig. 6

Lipman-Pearson Protein Alignment

Ktuple: 2; Gap Penalty: 4; Gap Length Penalty: 12

Seq1(1>129) PHTC.PRO	Seq2(1>150) EST2P.PRO	Similarity Index	Gap Number	Gap Length	Consensus Length
(3>85)	(1>80)	23.3	3	3	83

```
                   ↓10       ↓20       ↓30       ↓40       ↓50       ↓60       ↓70       ↓80
PHTC.PRO    FLHWLMSVYVVELLRSFFYVTETTFQKNRLFFYRKSVWSKLQSIGIRQHLKRVQLRDVSEAEVRQHREARPALLTSRLRF
            F: WL..  : .::::FFY TE.:   .  : ::R...W:KL :  I :.:K  L : ::.  .::. : :.:  S::R:
EST2P.PRO   FISWLFRQLIPKIIQTFFYCTEIS-STVTIVYFRHDTWNKLITPFIVEYFKTY-LVE-NNVCRNHNSYTLSNFNHSKMRI
                     ↑10       ↑20        ↑30       ↑40       ↑50         ↑60       ↑70

                   ↓90       ↓100      ↓110      ↓120
PHTC.PRO    IPKPDGLRPIVNMDYVVGARTFRREKRAERLTSRVKALFSVLNYERA
            IPK ..     :      GA .   .   E. .: :::  .:L:Y R.
EST2P.PRO   IPKKSNNEFRIIAIPCRGADEEEFTIYKENHKNAIQPTQKILEYLRN
              ↑80       ↑90       ↑100      ↑110      ↑120
```

Fig. 7

Alignment Workspace of Untitled, using Clustal method with PAM250 residue weight table

```
           -KFLXWLFXXLVVXLIRXFFYVTEXXXSXXXXXXYYRKXXWXKLXXXXIXXXLKXXXLXXVXEXEVRXHXXXXLX-FXXS
                     10        20        30        40        50        60        70        80
PHTC.PRO   AKFLHWLMSVYVVELLRSFFYVTETTFQKNRLFFYRKSVWSKLQSIGIRQHLKRVQLRDVSEAEVRQHREARPA-LLTS
P123.PRO   -KLLRWIFEDLVVSLIRCFFYVTEQQKSYSKTYYYRKNIWDVIMKMSI-ADLKKETLAEVQEKEV-EEWKKSLG-FAPG
EST2P.PRO  --FISWLFRQLIPKIIQTFFYCTEIS-STVTIVYFRHDTWNKLITPFIVEYFKTYL---VENNVCRNHNSYTLSNFNHS

           LRXIPKKXX--FRPIXXXXXXXXXX---TXXXEXXXXXLTXXXKXLX---XLXXXXXXXFXXX--FSVXNYXDXXKXXX
                     90        100       110       120       130       140       150       160
PHTC.PRO   LRFIPKPDG--LRPIVNMDYVVGAR---TFRREKRAERLTSRVKAL------------------FSVLNYERA
P123.PRO   LRLIPKKTT--FRPIMTFN----KK---IVNSDRKTTKLTTNTKLLNSHLMLKTLKNRMFKDPFGFAVFNYDDVMKKYE
EST2P.PRO  MRIIPKKSNNEFR-IIAIPCRGADEEEFTIYKENHKNAIQPTQKILE---YLRNKRPTSFTKI--YSPTQIADRIKEFK
```

Fig. 8

GTGCCTGCAG AGACCCGTCT GGTGCACTCT GATTCTCCAC TTGCCTGTTG CATGTCCTCG TTCCCTTGTT 70
TCTCACCACC TCTTGGGTTG CCATGTGCGT TTCCTGCCGA GTGTGTGTTG ATCCTCTCGT TGCCTCCTGG 140
TCACTGGGCA TTTGCTTTTA TTTCTCTTTG CTTAGTGTTA CCCCCTGATC TTTTTATTGT CGTTGTTTGC 210
TTTTGTTTAT TGAGACAGTC TCACTCTGTC ACCCAGGCTG GAGTGTAATG GCACAATCTC GGCTCACTGC 280
AACCTCTGCC TCCTCGGTTC AAGCAGTTCT CATTCCTCAA CCTCATGAGT AGCTGGGATT ACAGGCGCCC 350
ACCACCACGC CTGGCTAATT TTTGTATTTT TAGTAGAGAT AGGCTTTCAC CATGTTGGCC AGGCTGGTCT 420
CAAACTCCTG ACCTCAAGTG ATCTGCCCGC CTTGGCCTCC CACAGTGCTG GGATTACAGG TGCAAGCCAC 490
CGTGCCCGGC ATACCTTGAT CTTTTAAAAT GAAGTCTGAA ACATTGCTAC CCTTGTCCTG AGCAATAAGA 560
CCCTTAGTGT ATTTTAGCTC TGGCCACCCC CCAGCCTGTG TGCTGTTTTC CCTGCTGACT TAGTTCTATC 630
TCAGGCATCT TGACACCCCC ACAAGCTAAG CATTATTAAT ATTGTTTTCC GTGTTGAGTG TTTCTTTAGC 700
TTTGCCCCCG CCCTGCTTTT CCTCCTTTGT TCCCCGTCTG TCTTCTGTCT CAGGCCCGCC GTCTGGGGTC 770
CCCTTCCTTG TCCTTTGCGT GGTTCTTCTG TCTTGTTATT GCTGGTAAAC CCCAGCTTTA CCTGTGCTGG 840
CCTCCATGGC ATCTAGCGAC GTCCGGGGAC CTCTGCTTAT GATGCACAGA TGAAGATGTG GAGACTCACG 910
AGGAGGGCGG TCATCTTGGC CCGTGAGTGT CTGGAGCACC ACGTGGCCAG CGTTCCTTAG CCAGGGTTGG 980
CTGTGTTCCG GCCGCAGAGC ACCGTCTGCG TGAGGAGATC CTGGCCAAGT TCCTGCACTG GCTGATGAGT 1050
GTGTACGTCG TCGAGCTGCT CAGGTCTTTC TTTTATGTCA CGGAGACCAC GTTTCAAAAG AACAGGCTCT 1120
TTTTCTACCG GAAGAGTGTC TGGAGCAAGT TGC 1153

Fig. 9

CAGAGCCCTG GTCCTCCTGT CTCCATCGTC ACGTGGGCAC ACGTGGCTTT TCGCTCAGGA CGTCGAGTGG 70
ACACGGTGAT CTCTGCCTCT GCTCTCCCTC CTGTCCAGTT TGCATAAACT TACGAGGTTC ACCTTCACGT 140
TTTGATGGAC ACGCGGTTTC CAGGCACCGA GGCCAGAGCA GTGAACAGAG GAGGCTGGGC GCGGCAGTGG 210
AGCCGGGTTG CCGGCAATGG GGAGAAGTGT CTGGAAGCAC AGACGCTCTG GCGAGGGTGC CTGCAGAGAC 280
CCGCCTGGTG CACTCTGATT CTCCACTTGC CTGTTGCATG TCCTCGTTCC CTTGTTTCTC ACCACCTCTT 350
GGGTTGCCAT GTGCGTTTCC TGCCGAGTGT GTGTTGATCC TCTCGTTGCC TCCTGGTCAC TG 412

Fig. 10

GGGGTCCTGG GCCCACCCGG GCAGGACGCG TGGACCGAGT GACCGTGGTT TCTGTGTGGT GTCACCTGCC 70
AGACCCGCCG AAGAAGCCAC CTCTTTGGAG GGTGCGCTCT CTGGCACGCG CCACTCCCAC CCATCCGTGG 140
GCCGCCAGCA CCACGCGGGC CCCCCATCCA CATCGCGGCC ACCACGTCCC TGGGACACGC CTTGTCCCCC 210
GGTGTACGCC GAGACCAAGC ACTTCCTCTA CTCCTCAGGC GACAAGGAGC AGCTGCGGcC CTCCTTCCTA 280
CTCAGCTCTC TGAGGCCCAG CCTGACTGGC GCTCGGAGGC TCGTGGAGAC CATCTTTCTG GGTTCCAGGC 350
CCTGGATGCC AGGGACTCCC CGCAGGTTGC CCCGCCTGCC CCAGCGCTAC TGGCAAATGC GGCCCCTGTT 420
TCTGGAGCTG CTTGGGAACC ACGCGCAGTG CCCCTACGGG GTGCTCCTCA AGACGCACTG CCCGCTGCGA 490
GCTGCGGTCA CCCCAGCAGC CGGTGTCTGT GCCCGGGAGA AGCCCCAGGG CTCTGTGGCG GCCCCCGAGG 560
AGGAGGACAC AGACCCCCGT CGCCTGGTGC AGCTGCTCCG CCAGCACAGC AGCCCCTGGC AGGTGTACGG 630
CTTCGTGCGG GCCTGCCTGC GCCGGCTGGT GCCCCCAGGC CTCTGGGGCT CCAGGCACAA CGAACGCCGC 700
TTCCTCAGGA ACACCAAGAA GTTCATCTCC CTGGGGAAGC ATGCCAAGCT CTCGCTGCAG GAGCTGACGT 770
GGAAGATGAG CGTGCGGGAC TGCGCTTGGC TGCGCAGGAG CCCAGGTGAG GAGGTGGTGG CCGTCGAGGG 840
CCCAGGCCCC AGAGCTGAAT GCAGTAGGGG CTCAGAAAAG GGGGCAGGCA GAGCCCTGGT CCTCCTGTCT 910
CCATCGTCAC GTGGGCACAC GTGGCTTTTC GCTCAGGACG TCGAGTGGAC ACGGTGATCT CTGCCTCTGC 980
TCTCCCTCCT GTCCAGTTTG CATAAACTTA CG 1012

Fig. 11

```
GAATTCGCGG CCGCGTCGAC GTTTCAGGCA GCGCTGCGTC CTGCTGCGCA CGTGGGAAGC CCTGGCCCCG 70
GCCACCCCCG CGATGCCGCG CGCTCCCCGC TGCCGAGCCG TGCGCTCCCT GCTGCGCAGC CACTACCGCG 140
AGGTGCTGCC GCTGGCCACG TTCGTGCGGC GCCTGGGGCC CCAGGGCTGG CGGCTGGTGC AGCGCGGGGA 210
CCCGGCGGCT TTCCGCGCGC TGGTGGCCCA GTGCCTGGTG TGCGTGCCCT GGGACGCACG GCCGCCCCCC 280
GCCGCCCCCT CCTTCCGCCA GGTGTCCTGC CTGAAGGAGC TGGTGGCCCG AGTGCTGCAG AGGCTGTGCG 350
AGCGCGGCGC GAAGAACGTG CTGGCCTTCG GCTTCGCGCT GCTGGACGGG GCCCGCGGGG GCCCCCCCGA 420
GGCCTTCACC ACCAGCGTGC GCAGCTACCT GCCCAACACG GTGACCGACG CACTGCGGGG GAGCGGGGCG 490
TGGGGGCTGC TGCTGCGCCG CGTGGGCGAC GACGTGCTGG TTCACCTGCT GGCACGCTGC GCGCTCTTTG 560
TGCTGGTGGC TCCCAGCTGC GCCTACCAGG TGTGCGGGCC GCCGCTGTAC CAGCTCGGCG CTGCCACTCA 630
GGCCCGGCCC CCGCCACACG CTAGTGGACC CCGAAGGCGT CTGGGATGCG AACGGGCCTG GAACCATAGC 700
GTCAGGGAGG CCGGGGTCCC CCTGGGCCTG CCAGCCCCGG GTGCGAGGAG GCGCGGGGGC AGTGCCAGCC 770
GAAGTCTGCC GTTGCCCAAG AGGCCCAGGC GTGGCGCTGC CCCTGAGCCG GAGCGGACGC CCGTTGGGCA 840
GGGGTCCTGG GCCCACCCGG GCAGGACGCG TGGACCGAGT GACCGTGGTT TCTGTGTGGT GTCACCTGCC 910
AGACCCGCCG AAGAAGCCAC CTCTTTGGAG GGTGCGCTCT CTGGCACGCG CCACTCCCAC CCATCCGTGG 980
GCCGCCAGCA CCACGCGGGC CCCCCATCCA CATCGCGGCC ACCACGTCCC TGGGACACGC CTTGTCCCCC 1050
GGTGTACGCC GAGACCAAGC ACTTCCTCTA CTCCTCAGGC GACAAGGAGC AGCTGCGGCC CTCCTTCCTA 1120
CTCAGCTCTC TGAGGCCCAG CCTGACTGGC GCTCGGAGGC TCGTGGAGAC CATCTTTCTG GGTTCCAGGC 1190
CCTGGATGCC AGGGACTCCC CGCAGGTTGC CCCGCCTGCC CCAGCGCTAC TGGCAAATGC GGCCCCTGTT 1260
TCTGGAGCTG CTTGGGAACC ACGCGCAGTG CCCCTACGGG GTGCTCCTCA AGACGCACTG CCCGCTGCGA 1330
GCTGCGGTCA CCCCAGCAGC CGGTGTCTGT GCCCGGGAGA AGCCCCAGGG CTCTGTGGCG GCCCCCGAGG 1400
AGGAGGACAC AGACCCCCGT CGCCTGGTGC AGCTGCTCCG CCAGCACAGC AGCCCCTGGC AGGTGTACGG 1470
CTTCGTGCGG GCCTGCCTGC GCCGGCTGGT GCCCCCAGGC CTCTGGGGCT CCAGGCACAA CGAACGCCGC 1540
TTCCTCAGGA ACACCAAGAA GTTCATCTCC CTGGGGAAGC ATGCCAAGCT CTCGCTGCAG GAGCTGACGT 1610
GGAAGATGAG CGTGCGGGAC TGCGCTTGGC TGCGCAGGAG CCCAGGTGAG GAGGTGGTGG CCGTCGAGGG 1680
CCCAGGCCCC AGAGCTGAAT GCAGTAGGGG CTCAGAAAAG GGGGCAGGCA GAGCCCTGGT CCTCCTGTCT 1750
CCATCGTCAC GTGGGCACAC GTGGCTTTTC GCTCAGGACG TCGAGTGGAC ACGGTGATCT CTGCCTCTGC 1820
TCTCCCTCCT GTCCAGTTTG CATAAACTTA CGAGGTTCAC CTTCACGTTT TGATGGACAC GCGGTTTCCA 1890
GGCGCCGAGG CCAGAGCAGT GAACAGAGGA GGCTGGGCGC GGCAGTGGAG CCGGGTTGCC GGCAATGGGG 1960
AGAAGTGTCT GGAAGCACAG ACGCTCTGGC GAGGGTGCCT GCAGGGGTTG GCTGTGTTCC GGCCGCAGAG 2030
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TCAGGTCTTT CTTTTATGTC ACGGAGACCA CGTTTCAAAA GAACAGGCTC TTTTTCTACC GGAAGAGTGT 2170
CTGGAGCAAG TTGCAAAGCA TTGGAATCAG ACAGCACTTG AAGAGGGTGC AGCTGCGGGA GCTGTCGGAA 2240
GCAGAGGTCA GGCAGCATCG GGAAGCCAGG CCCGCCCTGC TGACGTCCAG ACTCCGCTTC ATCCCCAAGC 2310
CTGACGGGCT GCGGCCGATT GTGAACATGG ACTACGTCGT GGGAGCCAGA ACGTTCCGCA GAGAAAAGAG 2380
GGTGGCTGTG CTTTGGTTTA ACTTCCTTTT TAAACAGAAG TGCGTTTGAG CCCCACATTT GGTATCAGCT 2450
TAGATGAAGG GCCCGGAGGA GGGGCCACGG GACACAGCCA GGGCCATGGC ACGGCGCCAA CCCATTTGTG 2520
CGCACGGTGA GGTGGCCGAG GTGCCGGTGC CTCCAGAAAA GCAGCGTGGG GGTGTAGGGG GAGCTCCTGG 2590
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TTCAGAGTCT CAAAAGCTGG GACCCTCAGG GCCAAATGGG GCCACACCTT GTCCTGGAAG AAATCATGGT 2800
CCACTTCCAG GTTCGCCGGG TCCGGTTCTT CCTGCTCAGT GGGGCTACGA CCACCTAGGT AGTTGCTACC 2870
TAATCCTTCC CGGCGAAAAT AGGCTCCACT GGTGTCTGCA ACAAGCGGAG TCTCTAGGCC TGGTCCCTGG 2940
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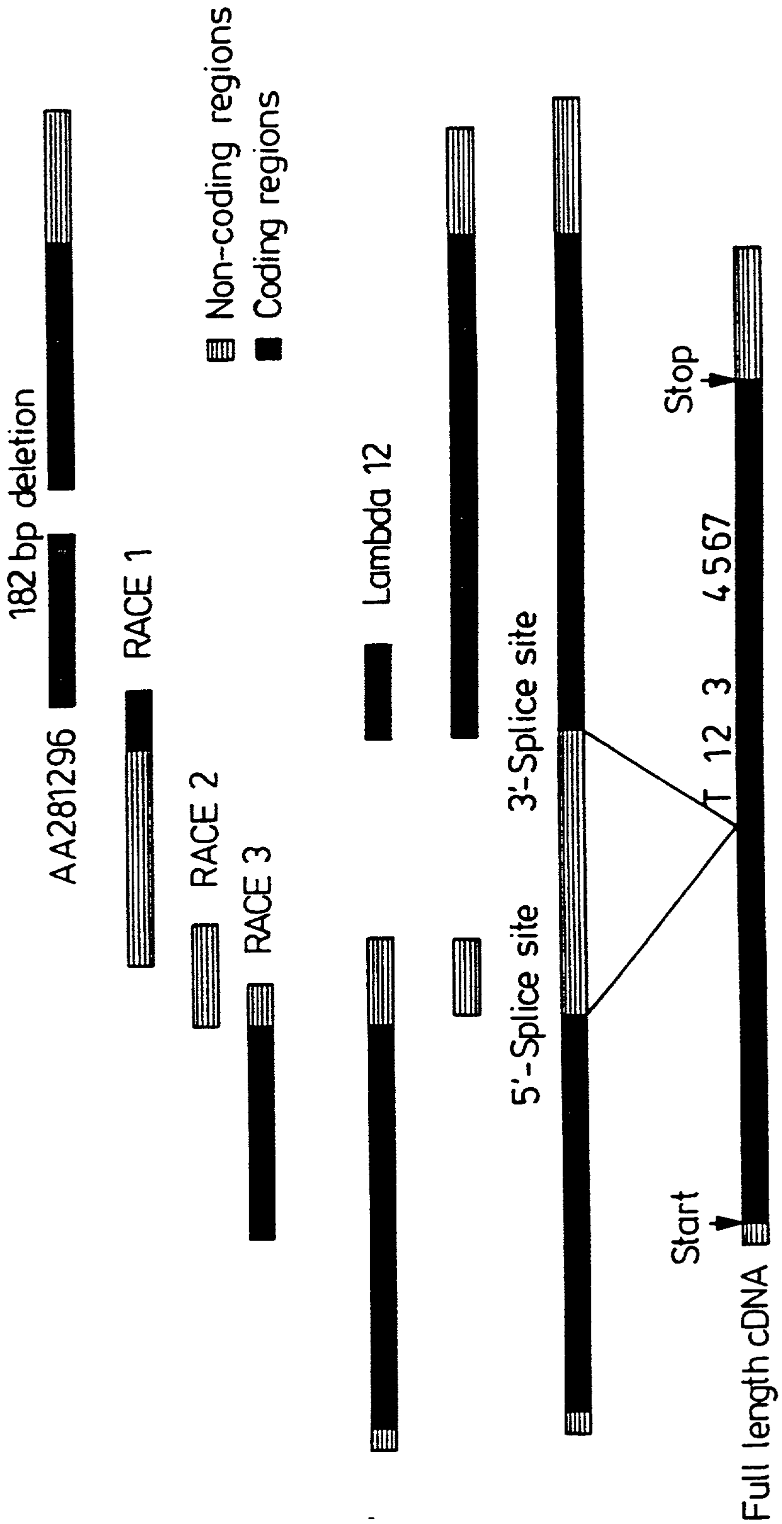
Fig.12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Coding regions
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182 bp deletion
RACE 1
RACE 2
RACE 3
Lambda 12
5'-Splice site
3'-Splice site
T 12 3 4567
Start
Stop
Full length cDNA

09/424686

Fig. 13

Telomerase motif

	Telomerase motif (553–565)
hTC	VVELLRSFFYVTE
Euplotes	VVSLIRCFFYVTE

	RT1 (619–626)	RT2 (630–641)	RT3 (704–720)
RT consens.	p-hh-h-K	hR-h-------K	h--hDh---Y--h
hTC	SRLRFIPK	LRPIVNMDYVVG	PELYFVKVDVTGAYDTI
Euplotes	GKLRLIPK	FRPIMTFNKKIV	PELYFMKFDVKSCYDSI

	RT4 (827–839)	RT5 (863–872)	RT6 (895–902)	RT7 (913–918)
RT consens.	h---hPQG---SP	h--YhDDhhh	Gh-h---K	hLG--h
hTC	QCQGIPQGSILST	LLRLVDDFLL	GCVVNLRK	ALGGTA
Euplotes	QTKGIPQGLCVSS	LMRLTDDYLL	GFKFNMKK	KFAKYG

Fig. 14

CCGGAAGAGT GTCTGGAGCA AGTTGCAAAG CATTGGAATC AGACAGCACT TGAAGAGGGT GCAGCTGCGG 1853
GAGCTGTCGG AAGCAGAGGT CAGGCAGCAT CGGGAAGCCA GGCCCGCCCT GCTGACGTCC AGACTCCGCT 1923
TCATCCCCAA GCCTGACGGG CTGCGGCCGA TTGTGAACAT GGACTACGTC GTGGGAGCCA GAACGTTCCG 1993
CAGAGAAAAG AGGGCCGAGC GTCTCACCTC GAGGGTGAAG GCACTGTTCA GCGTGCTCAA CTACGAGCGG 2063
GCGCGGCGCC CCGGCCTCCT GGGCGCCTCT GTGCTGGGCC TGGACGATAT CCACAGGGCC TGGCGCACCT 2133
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TGCGTGCGTC GGTATGCCGT GGTCCAGAAG GCCGCCCATG GGCACGTCCG CAAGGCCTTC AAGAGCCACG 2343
TCTCTACCTT GACAGACCTC CAGCCGTACA TGCGACAGTT CGTGGCTCAC CTGCAGGAGA CCAGCCCGCT 2413
GAGGGGTGCC GTCGTCATCG AGCAGAGCTC CTCCCTGAAT GAGGCCAGCA GTGGCCTCTT CGACGTCTTC 2483
CTACGCTTCA TGTGCCACCA CGCCGTGCGC ATCAGGGGCA AGTCCTACGT CCAGTGCCAG GGGATCCCGC 2553
AGGGCTCCAT CCTCTCCACG CTGCTCTGCA GCCTGTGCTA CGGCGACATG GAGAACAAGC TGTTTGCGGG 2623
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GCGAAAACCT TCCTCAGGAC CCTGGTCCGA GGTGTCCCTG AGTATGGCTG CGTGGTGAAC TTGCGGAAGA 2763
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CCTATTCCCC TGGTGCGGCC TGCTGCTGGA TACCCGGACC CTGGAGGTGC AGAGCGACTA CTCCAGCTAT 2903
GCCCGGACCT CCATCAGAGC CAGTCTCACC TTCAACCGCG GCTTCAAGGC TGGGAGGAAC ATGCGTCGCA 2973
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GGTGTGCACC AACATCTACA AGATCCTCCT GCTGCAGGCG TACAGGTTTC ACGCATGCGT GCTGCAGCTC 3113
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TGGAAGGGAC AGGAGCTGTC TGGGAGCTGC CATCCTTCCC ACCTTGCTCT GCCTGGGGAA GCGCTGGGGG 3463
GCCTGGTCTC TCCTGTTTGC CCCATGGTGG GATTTGGGGG GCCTGGCCTC TCCTGTTTGC CCTGTGGTGG 3533
GATTGGGCTG TCTCCCGTCC ATGGCACTTA GGGCCCTTGT GCAAACCCAG GCCAAGGGCT TAGGAGGAGG 3603
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Fig. 15

Fig. A

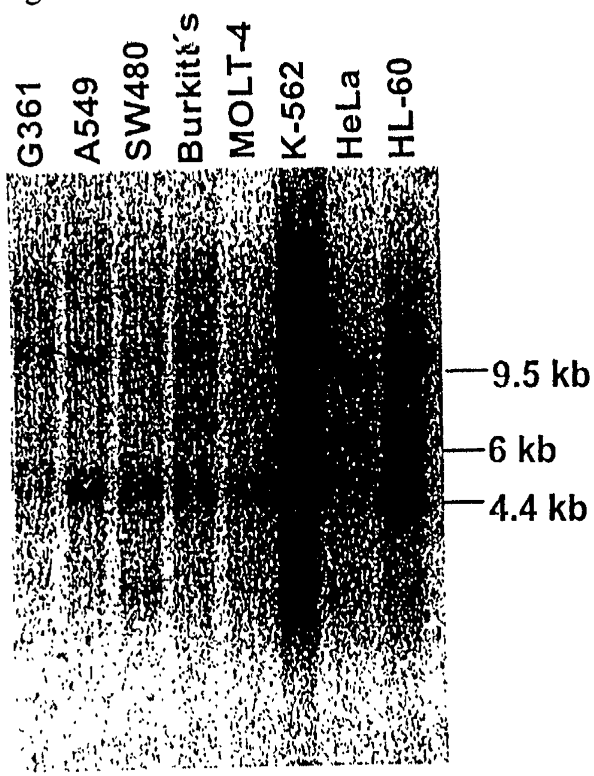


Fig. B

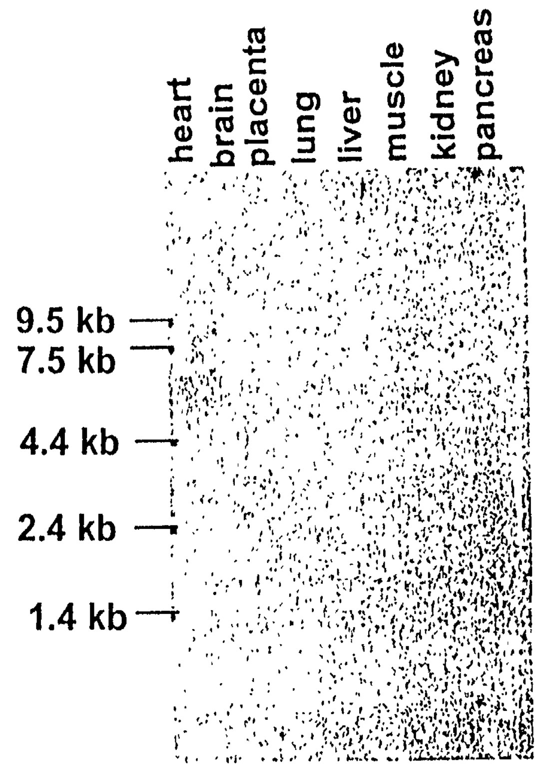
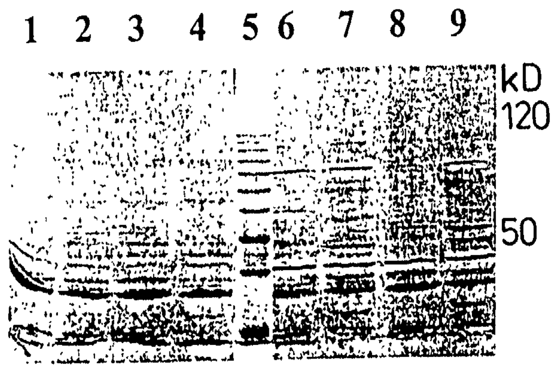


Fig. 16

Fig. A



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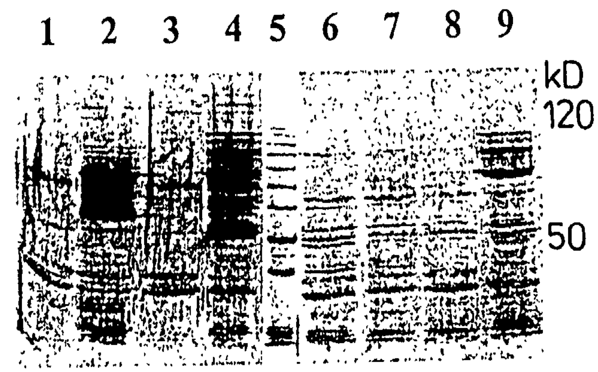
-	+	-	+		-	+	-	+
anti-B pre-immun					anti-C pre-immun			

Fig. B`



pMAL-EST	pMAL-EST	PMAL AI	pMAL AI	M	pMAL-EST	pMAL-EST	pMAL AI	pMAL AI
-	+	-	+		-	+	-	+
anti-B 1° immun.					anti-C 1° immun.			

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Fig. 17

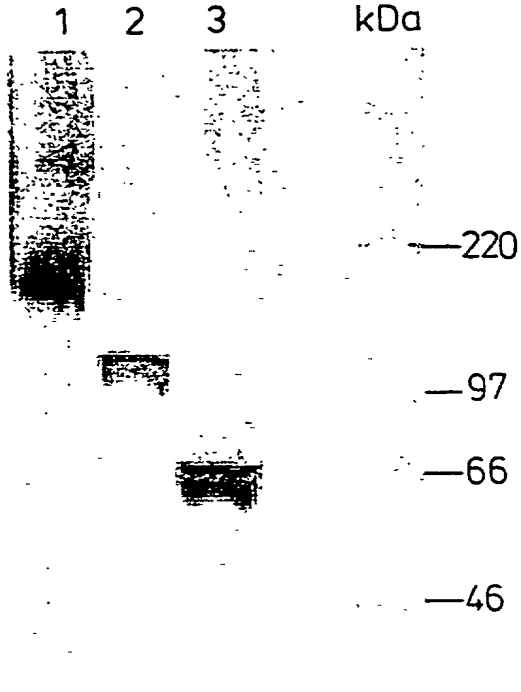

Fig. 19

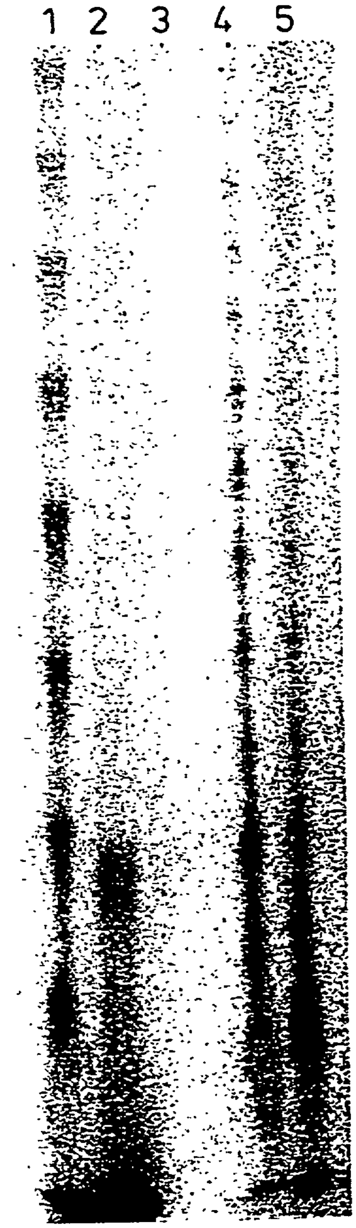

Fig. 18

COMBINED DECLARATION AND POWER OF ATTORNEY	ATTORNEY DOCKET NO

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name. I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought
on the invention entitled

HUMAN CATALYTIC TELOMERASE SUB-UNIT AND ITS DIAGNOSTIC AND THERAPEUTIC USE

the specification of which is attached hereto,

or was filed on **June 9, 1998**

as a PCT Application Serial No. **PCT/EP98/03468**

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims.

I acknowledge the duty to disclose information which is material to the patentability of this application in accordance with Title 37, Code of Federal Regulations, §1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

Prior Foreign Application(s), the priority(ies) of which is/are to be claimed:

197 26 329.1 (Number)	**Germany** (Country)	**June 20, 1997** (Month/Day/Year Filed)
198 13 274.3 (Number)	**Germany** (Country)	**March 26, 1998** (Month/Day/Year Filed)
198 16 496.3 (Number)	**Germany** (Country)	**April 14, 1998** (Month/Day/Year Filed)

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose the material information as defined in Title 37, Code of Federal Regulations, §1.56 which occured between the filing date of the prior application and the national or PCT international filing date of this application:

(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)
(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith:

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5-D

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RESIDENCE		CITIZENSHIP
POST OFFICE ADDRESS		

FULL NAME OF SEVENTH INVENTOR	INVENTOR'S SIGNATURE	DATE
RESIDENCE		CITIZENSHIP
POST OFFICE ADDRESS		

FULL NAME OF EIGHTH INVENTOR	INVENTOR'S SIGNATURE	DATE
RESIDENCE		CITIZENSHIP
POST OFFICE ADDRESS		

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Le A 32 486-US